Maternal Diet, Arsenic Exposure, and Pregnancy Outcomes in Bangladesh

MATERNAL DIET, ARSENIC EXPOSURE, AND PREGNANCY OUTCOMES IN BANGLADESH

PI-I LIN

A Dissertation Submitted to the Faculty of

The Harvard T.H. Chan School of Public Health

in Partial Fulfillment of the Requirements

for the Degree of *Doctor of Science*

in the Department of Environmental Health

Harvard University

Boston, Massachusetts.

November 2017

Dissertation Advisor: Dr. David Christiani Pi-I Lin

Maternal Diet, Arsenic Exposure, and Pregnancy Outcomes in Bangladesh

Abstract

This study investigated the relationship between maternal diet, arsenic exposure and birth outcomes in the Bangladeshi administrative regions of Sirajdikhan and Pabna Sadar. First, the study assessed the validity of a dish-based food frequency questionnaire (FFQ) by comparing it with two 3-day food diaries (FD). Food and nutrient intakes measured by FFQ and FD were compared using Pearson's and Spearman's correlation, paired *t*-test, percent difference, cross-classification, weighted Kappa, and Bland–Altman analysis. Results suggested that the FFQ exhibited good ability to assess and rank long-term dietary intake for most food groups and nutrients. Using this FFQ, the study then evaluated the relationship between long-term dietary habits and total arsenic concentration in toenail clippings. Associations between natural log-transformed consumption of individual food items and temporally matched natural log-transformed toenail arsenic concentration were quantified using general linear models that accounted for arsenic in the primary drinking water source and other potential confounders. Toenail arsenic was positively associated with consumption of several vegetable, fish and meat items and was negatively associated with consumption of rice, cereal, fruits, and milk based food items. The last part of the study used causal mediation analysis to investigate the causal pathway between diet, arsenic and fetal growth factors associated with birth weight. Mediating analysis showed significant natural indirect effects by toenail arsenic in the relationship between absolute fat, carbohydrate and fiber intake on gestational age at birth, specifically 3% (95% CI: 1%-6%)

of the association between carbohydrate intake and gestational age at birth was mediated by change in toenail arsenic level, while the mediating effect was 6% (95% CI: 1%-9%) and 10% (95% CI: 4%-13%) for absolute fat and fiber intake, respectively. After adjusting for total energy, no significant mediating effect was found, suggesting the absolute amount of arsenic exposure rather than the arsenic level in relationship to total energy intake was a more important factor to consider when understanding the negative implication of arsenic on fetal growths.

Table of Contents

List of Figures with Captions

List of Tables with Captions

Acknowledgements

Firstly, I would like to express my sincere gratitude to my advisor Professor David Christiani for the continuous support of my doctoral study and related research, for his patience, motivation, and immense knowledge. His guidance helped me in all the time of research and writing of this dissertation. I could not have imagined having a better advisor and mentor for my doctoral study.

Besides my advisor, I would like to thank the rest of my dissertation research committee: Dr. Joseph Allen and Dr. Emily Oken for their insightful comments and encouragement, but also for the hard question which incented me to widen my research from various perspectives.
My sincere thanks also goes to the research group at Professor David Christiani's lab at Harvard T.H. Chan School of Public Health as well as staff and field workers at the Nutrition Department of Dhaka Community Hospital, Bangladesh for their assistant and team work. I also gratefully acknowledge our study participants in Bangladesh, without whom the study would not be possible.

I thank my fellow classmate, Sabri Bromage, for the stimulating discussions on nutritional epidemiology and for reviewing my manuscripts. Also I thank the staff in the following institutions: Department of Environmental Health at Harvard T.H. Chan School of Public Health for the administrative supports, U.S. National Institutes of Health and Research Center for Environmental Medicine at Kaohsiung Medical University for research funding.

Last but not the least, I would like to thank my family and friends for supporting me spiritually throughout writing this thesis and my life in general.

Introduction

Arsenic is a human toxicant with significant public health impact. Chronic arsenic exposure is found to be associated with skin changes and increased risk for skin, lung, bladder and kidney cancer. Adverse birth outcomes from maternal arsenic exposure have been a growing concern but insufficient evidence is available to establish the causal association. Exposure to arsenic may occur by a variety of routes. In areas such as Bangladesh where high arsenic concentration is found in drinking water, a significant amount of inorganic arsenic exposure comes from water. In other areas without high level of arsenic in the drinking water, the majority of the daily intake of arsenic comes from food. Although efforts have been devoted to solve the arsenic problem from drinking water, arsenic exposure from food is often overlooked. Our research group found that in areas in Bangladesh where water arsenic concentration was lower than the national standard for water arsenic level (50μg/L), dietary arsenic could still contribute significantly to the ingested dose. More evidence indicates that diet alone can be a significant source of arsenic exposure. However, the effects of arsenic from diet and from drinking and water sources have not been differentiated among pregnant women. Our research team has established a reproductive cohort in Bangladesh since 2008 that recruited 1613 mother infant pairs. Preliminary results indicated that maternal arsenic exposure was associated with low birth weight in Bangladesh. However, the analysis did not consider diet and nutritional intake, which could be an important effect modifier, especially among those who had low well water arsenic source. Diet and nutrition are important determinants of fetal and maternal health. This study

aims to investigate the relationships between maternal diet, maternal arsenic exposure, and birth outcomes. First, we assessed the validity of the food frequency questionnaire used in this prospective cohort (Chapter 1) to ensure the dietary data are valid. Then, we examined the association between dietary intake and maternal toenail arsenic concentration (Chapter 2) and evaluated the potential mediating role of arsenic in the relationship between maternal diet and birth outcomes (Chapter 3).

Chapter 1. Validation of a Dish-Based Semiquantitative Food Questionnaire in Rural Bangladesh

Abstract: A locally validated tool was needed to evaluate long-term dietary intake in rural Bangladesh. We assessed the validity of a 42-item dish-based semi-quantitative food frequency questionnaire (FFQ) using two 3-day food diaries (FDs). We selected a random subset of 47 families (190 participants) from a longitudinal arsenic biomonitoring study in Bangladesh to administer the FFQ. Two 3-day FDs were completed by the female head of the households and we used an adult male equivalent method to estimate the FD for the other participants. Food and nutrient intakes measured by FFQ and FD were compared using Pearson's and Spearman's correlation, paired *t*-test, percent difference, cross-classification, weighted Kappa, and Bland–Altman analysis. Results showed good validity for total energy intake (paired *t*-test, $p < 0.05$; percent difference <10%), with no presence of proportional bias (Bland–Altman correlation, $p > 0.05$). After energy-adjustment and de-attenuation for within-person variation, macronutrient intakes had excellent correlations ranging from 0.55 to 0.70. Validity for micronutrients was mixed. High intraclass correlation coefficients (ICCs) were found for most nutrients between the two seasons, except vitamin A. This dish-based FFQ provided adequate validity to assess and rank long-term dietary intake in rural Bangladesh for most food groups and nutrients, and should be useful for studying dietary-disease relationships.

Keywords: food frequency questionnaire; food diary; duplicate food sample; validation study; adult male equivalent; Bangladesh

1.1. Introduction

Understanding dietary habits is important for studying the development and progression of chronic illnesses. Accurate assessment of dietary habits is crucial in large, prospective epidemiological studies to provide a holistic understanding of health status. Having reliable dietary data allows researchers to examine the relationship of variations in food and nutrient intakes with the susceptibility to adverse effects from environmental factors and diseases. In Bangladesh, limited dietary intake data is available in most epidemiological studies; as a result, most studies had not accounted for individual variations in dietary intakes. Many national surveys employed a labor-intensive 24-h recall method to collect data at the household level [1–7], but individual habitual dietary data is lacking. Although household data are useful for designing nutrition interventions, more expensive and long-term dietary surveillance is needed to study and prevent chronic diseases.

The food frequency questionnaire (FFQ), which is comprised of a predefined list of food and beverages with response categories to indicate consumption frequency, is a low-cost tool and has been the most widely used method for ranking participants' dietary and nutrient intake in epidemiologic studies [8]. The validity of a FFQ in measuring relative dietary intake is important. Only one FFQ in Bangladesh using a food-based approach had previously been validated [9]. In rural Bangladesh, where typical dietary patterns are characterized by various mixed food dishes rather than single food ingredients, using this food-based FFQ created problems of respondent fatigue and poorer quality of reported information [10–12]. In addition, the previously validated FFQ used food composition tables from the US and Indian nutrient databases to calculate nutrient values, which likely did not reflect the most accurate nutrient values of locally produced food, as food composition varies from country to country depending

on the species of plants and animals, agricultural technology, climactic condition, processing, and storage circumstances [10].

In this study, we employed a dish-based semi-quantitative FFQ previously developed for a longitudinal study investigating arsenic exposure and biomarker responses in Bangladesh [13,14] and used the 2013 Food Composition Table in Bangladesh to calculate nutrient intakes [15]. We assessed the validity of the dish-based FFQ by comparing the intakes calculated using the FFQ with intakes measured by two 3-day weighed food diaries (FDs). FD has been acknowledged as an appropriate reference for FFQ validation [10,16] since FD does not share common measurement errors with the FFQ, such as recall bias and errors from the conception of serving size and interpretation of questions [10]. The examination of the validity employed a multifaceted approach, utilizing six statistical tests, including Spearman's rank-order correlation, paired *t*-test, percent difference, cross-classification, weighted Kappa coefficient, and Bland–Altman analysis [17].

1.2. Materials and Methods

1.2.1. Participant Selection

We invited 248 subjects, from 47 households, concurrently enrolled in a longitudinal arsenic biomonitoring study in rural Pabna, Bangladesh, from 2001–2005 [13,14] to participate in the FFQ study. The recruitment process has been previously described [18]. One hundred and ninety subjects agreed to participate and provided written informed consents (Figure S1-1). All subjects completed one individual FFQ and the female head of the household in charge of food preparation completed two 3-day FDs. The sample size of 190 had greater than 95% power to detect matched pair mean difference in intake of any food or nutrient larger than 0.27 ($\alpha = 0.05$,

two-tails). The study was approved by the Human Subjects Committees of the Dhaka Community Hospital and the Harvard T.H. Chan School of Public Health (P10565/0204 MOLE, approved 8 November 2001).

1.2.2. FFQ

A dish-based semi-quantitative FFQ was previously developed to assess subjects' habitual dietary intake over the past 12 months. Development was based on a prior questionnaire used in a national-level nutritional survey conducted in Bangladesh and followed recommended guidelines on FFQ development [10,16]. Through focus group discussion with local community residents and dieticians from the Dhaka Community Hospital, the FFQ was constructed using commonly consumed dishes in Bangladesh from five major categories, including cereal and bread-based dishes; vegetable-based dishes; legume and pulse-based dishes; fish, poultry, meat, and egg-based dishes; and milk-based dishes. The dishes that contributed to each of the categories are listed in Table S1-1. Supplement and fluid consumption was also assessed. The final FFQ consisted of 42 dishes which were accepted based on the frequency of consumption and considered to make a substantial contribution to nutrient intakes of rural Bangladeshi adults. The FFQ assessed quantity, portion size, and frequency for each item consumed. Five frequency options were used: 'daily', 'weekly', 'monthly', 'yearly', and 'never'. The pre-specified portion sizes included large plate, medium plate, small plate, large bowl, medium bowl, small bowl, glass, cup, large spoon, small spoon, and piece. Visual aids for portion sizes were provided using locally used plates, bowls, cups, and serving utensils. Trained interviewers guided participants to ensure complete responses. All subjects completed one individual FFQ. The FFQ was administered in January 2004, at least one month before the collection of the FD.

1.2.3. Weighed FD

We instructed the female head of each household to keep a written FD at the time of consumption with quantities and portion sizes of all foods and beverages consumed at the time of consumption for three consecutive days in both winter (February–March 2004) and summer (June–August 2004), with one weekend day included in each period. We instructed the subject to report portion sizes using the same portion size option as specified in the FFQ, including large plate, medium plate, small plate, large bowl, medium bowl, small bow, glass, cup, large spoon, small spoon, and piece. We also asked subjects if the diet recorded each day reflected their usual diet or not. Concurrent with the FD collection, the female heads of the households provided a duplicate food sample (DFS) of each portion consumed and was reimbursed with $9 USD compensation after each sampling period (summer and winter). One field team member visited each participant at midday and in the evening to weigh DFS collected from morning to midday and from midday to evening, respectively. Detailed methods on the process of DFS have been described previously [13,14]. Measured food weights were used to calculate portion sizes for food dishes recorded in the FD of the female head of the household, whereas food weights for other family members were estimated by multiplying the consumption of all foods from the female head of the household by each other family member's respective adult male equivalent (AME). The AME is an expression of the relative estimated consumption of foods, based on the relation between an individual's predicted caloric requirement and those of an adult male [19]. Adult male equivalents (AMEs) have previously been calculated for different age and sex groups in Bangladesh using data from the 2010 Household Income and Expenditure Survey (HIES) [20], following the methodology of Bermudez *et al*. [21].

1.2.4. Analysis of Food and Nutrient Intake

Two trained technicians double-entered the primary data from FFQs and FDs to ensure accuracy. Daily nutrient intakes were calculated based on the 2013 Food Composition Table for Bangladesh [15]. The Food Composition Table includes nutrients of 328 food items and nutrient compositions of 27 single ingredient and 11 multi-ingredient recipes. For dishes in the FFQ that were not available in the Food Composition Table, their nutrient compositions were calculated based on nutrition values of the cooked ingredients according to average weighed recipes provided by local dietitians at DCH using the nutrient retention factors (RF) and yield factors (YF) provided in the 2013 Bangladesh Food composition Table. The mixed recipe calculation method was used, specifically, the YF is applied at the recipe level and RF was applied at the ingredient level [22]. Weighed recipes for food dishes were collected from 20 households in the study area and average recipes were calculated per 100 g for each food dish in different seasons. Average daily food intake assessed by FFQ was calculated using the formula:

$$\text{Intake}_{FFQ_i} = \sum (q_{ij} \times f_{ij} \times p_j) \quad (1)$$

where q is the reported quantity, f is the reported frequency per day, p is the pre-specified portion size in grams, for subject i and dish j. Average daily food intake assessed by FD was calculated using the formula:

$$\text{Intake}_{FD_i} = \frac{\sum (q_j \times mp_j)}{n} \times \frac{AME_i}{AME_k}) \quad (2)$$

where q is the recorded quantity in the FD, mp is the measured food weight in gram for dish j, AME is the adult male equivalent consumption for household members in Bangladesh based on age and sex [20] (previously calculated for different age and sex groups of the Bangladeshi

population using methodology of Bermudez *et al*. [21]), *n* is the number of days the FD was collected, and *k* is the person who filled out the FD in the household to which *i* subjects belonged. Food group intake was calculated by directly adding the consumption of each dish by food category, e.g., 'grain, cereal, and bread-based dish', 'vegetable-based dish', etc. Nutrient intakes and energy intake were calculated by multiplying the nutrient and energy values per 100 g of food adjusted for inedible waste from the Food Composition Table for Bangladesh [15]. The contribution from each food dish was then added for each participant to create the total nutrient and energy intakes.

1.2.5. Statistical Analysis

Demographic characteristics of the study population were tabulated. Food and nutrient intakes not normally distributed, as indicated by the Shapiro–Wilk test, were logarithmically transformed using the formula log(x + 1) to achieve a normal distribution. Body mass index (BMI) was calculated for participants aged 20 years of age or older using the formula weight (kg)/height squared (m^2). For participants under 20 years of age, we calculated age- and sex-specific BMI Z-scores using the WHO Growth Reference Charts [23]. FFQ responses with three missing components (missing quantity, missing frequency, and missing portion size) were imputed with the null value (no consumption). Responses with one or two missing components were imputed with the sample median conditioning on the values of the non-missing, e.g., a response with reported quantity of '2', frequency of 'daily', and missing portion size was imputed with the median portion size of samples reporting quantity of '2' and frequency of 'daily'.

Seasonal variations were tested by the Shrout–Fleiss intraclass correlation coefficient (ICC) between the winter and summer average dietary intakes derived from the two 3-day FD

[24]. The ICC, which is defined as a ratio of between-person variance to total variance, ranges from 0–1 and can be an indicator of reproducibility of dietary intake between the two seasons. Values from 0–0.25 indicate poor reproducibility; 0.25–0.4 indicate low reproducibility; 0.4–0.6 indicate fair reproducibility; 0.6–0.75 indicate good reproducibility; and 0.75–1.0 indicate excellent reproducibility [25,26].

To quantify the accuracy of the two 3-day FD in capturing individual's intake level, the unobservable (hypothetical) correlation coefficient (r_h) between observed and true mean nutrient intakes of the population during the period of observation was calculated using the following formula [27]:

$$r_h = \sqrt{\frac{D}{D + (S_w^2/S_b^2)}} \quad (3)$$

where S_w is the observed within-subject variance, S_b is the observed between-subject variance, and D is the number of days of FD collected for each participant.

The validity of the FFQ was assessed by comparing the intake of each nutrient/food group estimated from the FFQ with that estimated from the average of the two 3-day FD. Means and standard deviation (SD) of intakes for each nutrient and food group, as obtained from the FFQ and the FD, were computed separately, and means were compared using paired *t*-tests. Differences, ratios, and percent differences between mean values obtained with the FFQ and the FD were calculated to assess the level of agreement and direction of error [28,29]. Pearson's product moment correlation and Spearman's rank-order correlation were calculated to measure the strength and direction of the association. Since total energy intake can introduce extraneous variation in recorded food intake, intake estimates were adjusted for total energy intake using the residual method [30]. Briefly, residuals, which were derived from the model's food or nutrient

intakes regression on total energy intake, were standardized to the predicted log-transformed dietary intake of a subject with the average total energy intake of all subjects in the study. Energy-adjusted correlations were de-attenuated for within-person variability in measurements, which tends to reduce the correlation coefficient toward zero [31]. The deattenuation was performed using the formula:

$$R = \sqrt{r(1 + \lambda_x/n_x} \quad (4)$$

in which λ_x represents the ratio of the within- and between-person variances for x, and n_x represents the number of replicates for the x variable [10]. For this study, $n = 3$. Within- and between-person variations were calculated by one-way ANOVA for food and nutrient intakes estimated by the two 3-day FD.

In addition to using Spearman's correlation, FFQ's ability to rank participants correctly was assessed by cross-classification of energy-adjusted nutrient intakes into quintiles. Subjects that were assigned by the FFQ to the opposed extreme quintile of intakes based on their responses in the FD were considered grossly misclassified. The percentage of agreement indicated subjects with intakes ranked in the same quintile, though the agreement may occur by chance [32]. To assess the agreement accounting for chance, we used the weighted kappa statistic (κ_w) with Cicchetti–Allison weights, which measured the inter-rater agreement of categorical items accounting for natural ordering of categories [33–35].

Bland–Altman plots with differences between the measurements (y-axis) against the mean of the two measures (x-axis) for each subject were analyzed to assess the presence of proportional bias, as well as the direction of the bias between the FFQ and two 3-day FD for all nutrients and food intakes [32,36–38]. We also calculated the Bland–Altman Spearman correlation coefficient between the mean of the two methods and the mean difference of the two

methods to indicate the association between the size of the error, the presence of proportional bias, and the direction thereof.

We performed a sensitivity analysis restricted to only the FDs from the 47 female heads of household to assess the validity of the FFQ using Pearson and Spearman correlation. The correlations were also adjusted for energy intake using the residual method and the deattenuated correlations were calculated accounting for within-person variability in measurements.

Results were considered statistically significant at a two-tailed α level of 0.05. Statistical analysis was performed by using SAS software (release 9.4; SAS Institute Inc., Cary, NC, USA).

1.2.6. Integrative Interpretation of Statistical Outcomes

Multiple statistical tests were implemented to provide comprehensive evaluations on the various facets of validity. For the ease of visualization, results for the six statistical tests were color-coded based on levels of validity using criteria proposed by reviews of validity on dietary assessment methods [17]. Specifically, a correlation coefficient ≥0.40 was considered good, 0.20–0.39 was considered acceptable, and <0.20 was considered poor. Paired *t*-test of the mean intake was considered good when $p > 0.05$ and poor if $p \leq 0.05$. Percentage difference of the mean intake of 0.0%–10.9% was considered good, 11.0%–20.0% was considered acceptable, and >20.0% was considered poor. Cross-classification with ≤10% in opposite quintile was considered good and >10% was considered poor.

Weighted Kappa statistics of ≥0.61 was consider good, 0.15–0.59 was consider acceptable, and <0.15 was considered poor. A Bland–Altman correlation coefficient with a *p*-value > 0.05 was considered good, whereas *p*-value ≤ 0.05 was considered poor.

1.3. Results

All of the 190 subjects had one FFQ and two 3-day FDs. None of the FDs were collected on days of unusual diet (e.g., religious holiday). Table 1-1 presents the socio-demographic and lifestyle characteristics of the study subjects. Females comprised 54.21% of the study subjects. The cohort averaged 31.5 ± 14.2 years of age and a median BMI of 19.5 kg/m^2. Most of the participants had education levels lower than primary education. Housewives comprised 40.53% of the participants, 16.32% were students, 8.42% were factory labors, and 8.42% were businessmen. There was no missing data for the sex, age, BMI, or education variables. Fourteen percent of the job type category was missing values. Quantity values were missing in 0.06%, frequency values were missing in 0.37%, and portion size values were missing in 0.78% of the FFQ responses, before imputation.

Table 1-1. Socio-demographic characteristics of study participants.

Variables *	**n**	**%**
Sex (n = 190)		
Male	87	45.79
Female	103	54.21
Age (years) (n = 190)		
<10	5	2.6
11–20	53	27.9
21–30	41	21.6
31–40	45	23.7
41–50	29	15.3
51–60	10	5.3
>60	7	3.7
Median	30.0	
Mean	31.3	
SD	14.7	
Job Type (n = 190)		
Farmer	2	1.0
Agricultural labor	1	0.5
Factory labor	16	8.4
Businessman	16	8.4
Craftsman	0	0.0
Office worker	4	2.1
Housewife	77	40.5
Student	31	16.3
Jobless	7	3.7
Others	10	5.3
missing	26	13.7
BMI Z-score (for age 5–19 years, n = 48)		
1 SD	5	10.4
0 SD	5	10.4
−1 SD	12	25.0
−2 SD	13	27.1
−3 SD	7	14.6
−4 SD	6	12.5
BMI (kg/m^2, for age >20 years, n = 142)		
<18.1	30	21.1
18.1–20.5	39	27.5
>20.5	73	51.4
Median	20.8	
Mean	20.9	
SD	3.6	
Education (for age >20 years, n = 142)		
Illiterate	25	17.6
Able to write	50	35.2
Primary education	24	16.9
Secondary education	21	14.8
Higher secondary education	14	9.9
College/graduate	4	2.8
Post-graduate education	4	2.8
Missing	0	

* Children aged 5–19 years were included in the age, sex, and job type values, but were not included in the BMI and education values; children's BMI values were separately presented as BMI Z-score using the WHO Growth Reference Charts [23]. BMI=body mass index (kg/m^2); SD=Standard Deviation.

1.3.1. Seasonal Variability

Table 1-2 presents the ICCs between food and nutrient intakes of the two 3-day FD. ICC for daily average energy intake was high (0.80, $p < 0.001$), suggesting similar energy intake between different seasons. Intakes of beverages (ICC = 0.82, $p < 0.001$) and grain, cereal, and bread-based dishes (ICC = 0.78, $p < 0.001$) had the least seasonal variation. Intake of fruits (ICC = 0.24, $p < 0.001$) and legume- and pulse-based dishes (ICC = 0.20, $p < 0.001$) had large seasonal variations. Macronutrient intakes had small seasonal variations. Intake of vitamin A had the highest seasonal variation among all nutrient intakes (ICC = 0.38, $p < 0.001$).

1.3.2. Correlation of FD with True Intake

The unobservable (hypothetical) correlation coefficient (r_h) between intakes observed from the two 3-day FD and true mean nutrient intakes of the population during the period was 0.928. With $r_h > 0.90$, 80% of the individuals can be correctly classified into thirds of the distribution and less than 1% are grossly misclassified with 90% confidence [39]. The correlation between macro- and micronutrient intakes from the two 3-day FD and true mean nutrient intake ranged from 0.411 for zinc to 0.881 for protein (Table 1-2).

Table 1-2. Correlation of average daily food and nutrient intakes obtained from the two 3-day food diaries (FD) by the female head of the household, and the unobservable (hypothetical) correlation coefficient (r_h) between observed intake (from FD) and true mean nutrient intakes of the population during the study period (n = 47).

Dietary Intake [1]	ICC [2]	*p*-Value	r_h
Energy (kcal)	0.80	<0.001	0.928
Food Group			
Beverages	0.82	<0.001	0.942
Grain, Cereal, Bread based	0.78	<0.001	0.931
Milk based	0.53	<0.001	0.820
Vegetable based	0.53	0.66	0.825
Fish, Poultry, Meat, Egg based	0.50	<0.001	0.821
Fruits	0.24	<0.001	0.672
Legumes, Pulses, Seeds based	0.20	<0.001	0.616
Nutrients			
Alpha-carotene (mcg)	0.85	<0.001	0.748
Potassium (mg)	0.80	<0.001	0.844
Niacin, preformed (mg)	0.80	<0.001	0.767
Protein (g)	0.80	<0.001	0.881
Carbohydrate available (g)	0.79	0.02	0.851
Thiamin (mg)	0.78	<0.001	0.862
Phosphorus (mg)	0.78	<0.001	0.837
Riboflavin (mg)	0.76	<0.001	0.869
Ash (g)	0.74	<0.001	0.654
Calcium (mg)	0.73	<0.001	0.759
Vitamin B6 (mg)	0.73	<0.001	0.779
Total cryptoxanthin (mcg)	0.72	<0.001	0.712
Zinc (mg)	0.71	<0.001	0.411
Niacin equivalents (mg)	0.70	<0.001	0.834
Beta-carotene equivalents (mcg)	0.70	<0.001	0.748
Copper (mg)	0.70	<0.001	0.795
Niacin equivalents from tryptophan (mg)	0.69	<0.001	0.870
Folate (mcg)	0.69	<0.001	0.848
Total dietary fiber (g)	0.69	<0.001	0.643
Magnesium (mg)	0.68	<0.001	0.547
Vitamin E (mg)	0.66	<0.001	0.806
L-ascorbic acid (mg)	0.62	<0.001	0.768
Fat (g)	0.60	<0.001	0.813
Sodium (mg)	0.59	<0.001	0.729
Retinol (mcg)	0.53	0.19	0.751
Iron (mg)	0.50	<0.001	0.852
Vitamin D (mcg)	0.49	<0.001	0.787
Beta-carotene (mcg)	0.45	<0.001	0.709
Vitamin A (mcg)	0.38	<0.001	0.534

[1] Food intakes and nutrient intakes listed in the descending order of the intraclass correlation coefficient (ICC); [2] Calculated based on log-transformed intake values.

1.3.3. Validity

Table 1-3 presents the comparison of the average daily food group intakes assessed by FFQ and FD. Daily average consumption of grains, cereal, bread-based dishes, fish, poultry, meat, egg-based dish, milk-based dishes, and beverages were overestimated by the FFQ compared to the FD, whereas the FFQ underestimated consumptions of vegetable-based dishes and fruits. Pearson's and Spearman's rank correlation yielded similar correlation estimates, we only presented the results of the Pearson's correlation. The unadjusted Pearson's correlation coefficient ranges from 0.16 for vegetable-based dishes to 0.75 for fruits. After adjusting for total energy, Pearson's correlation coefficients increased for all food groups, except grain, cereal, bread-based dishes, and milk-based dishes. The deattenuated correlation corrected for within-person variation in the FD increased the correlation estimates of all food categories, especially for legume- and pulse-based dishes, indicating greater within-person variations as compared with between-person variations in the consumption of food in this category. Accounting for the effect of attenuation, the coefficients ranges from 0.25 for vegetable-based dishes to 0.90 for milk-based dishes.

Table 1-4 presents the average daily nutrient intakes and correlations between values derived from the FFQ and the FD. The trend of overestimation and underestimation of nutrient intakes was consistent with that of food categories. For instance, consistent with the overestimation of grain, cereal, and bread-based dishes, FFQ overestimated the carbohydrates available by 56% (FFQ:FD = 1.56); on the other hand, the underestimation of fruit intake concurred with lower L-ascorbic acid intake estimated by FFQ (FFQ:FD = 0.78). The unadjusted Pearson's correlations for nutrient intakes ranged from 0.08 for total cryptoxanthin to 0.38 for fat

and iron. Energy adjustments improved the correlations for most nutrients, especially for carbohydrates (0.25–0.54), fiber (0.34–0.45), and protein (0.36–0.51), which agreed with the energy-adjusted improvement in Pearson's coefficients found on vegetable based dishes (0.16–0.21) and legumes, pulses, and seed-based dishes (0.23–0.34). The energy-adjusted correlation coefficients for food or nutrient intakes between the FFQ and FD were all statistically significant ($p < 0.001$).

Analysis of Bland–Altman plots revealed that most macronutrient and micronutrient intakes did not show significant proportional bias and most of the points fell within the 95% limits of agreement (Figure S1-1), and no statistically significant association was observed between the differences and means for those nutrient intakes (Bland–Altman Spearman's correlation p-value > 0.05). Outliers were found for intakes of iron, sodium, zinc, vitamin A, and vitamin B6 (Bland–Altman plots not shown). However, Bland–Altman Spearman's correlations for these nutrient intakes were not statistically significant both before and after the removal of the outliers, suggesting no significant bias was present. The intake of carbohydrate, calcium, potassium, alpha-carotene, total cryptoxanthin, niacin equivalents, and L-ascorbic acid exhibited linear treads on Bland–Altman plots, among which all had a positive proportional bias except for carbohydrates, which had negative proportional bias, i.e., as the mean of carbohydrate intake measured by the two methods increases, the mean difference decreases.

The sensitivity analysis using only the two 3-day FD from the 47 female heads of the household and found similar results comparing to using the AME method. The overall analysis on the subset of 47 women did show stronger corrections for most intakes but, qualitatively, the results were similar. Data with only the 47 female heads of household are presented in Tables S1-2, Table S1-3, and Table S1-4.

1.3.4. Classification

Results of the cross-classification of food and nutrient intakes estimated by the FFQ and the FD are shown in Table 1-5. A high proportion of study participants (>70%) was correctly categorized into the same or adjacent quintile for estimates of all food groups and nutrient intakes. Intakes of fish, poultry, meat, egg-based dishes and legumes, pulses, and seed-based dishes had low proportions of grossly misclassified subjects (2.96% and 4.24%, respectively), whereas the intakes of beverages had a high proportion (11.21%). The weighted kappa coefficient matched the cross-classification finding, showing highest weighted kappa for fish, poultry, meat, and egg-based dishes (0.41, 95% CI: 0.35–0.46), and the lowest for beverages (0.07, 95%: 0.00–0.14). All nutrient intakes had a small proportion (average of 6.2%) of being grossly misclassified, among which total dietary fiber (0.90% grossly misclassified, weighted kappa 0.42, 95% CI: 0.33–0.47) was the lowest. Calcium, copper, phosphorus, L-ascorbic acid, beta-carotene, sodium, retinol, vitamin B6, and vitamin A had higher proportions of being grossly misclassified (>0.5%). However, none of the nutrient intakes had more than 10% in the opposite quintile, suggesting nutrient intakes estimated from FFQ and FD had a good agreement at the individual level [17].

Table 1-3. Degree of association and level of agreement between average daily food group intakes by FD and FFQ.

Food Group	Average Daily Consumption (Serving/Day)					Level of Agreement			Correlation Coefficient *		
	FD		FFQ			FFQ-FD [1]		FFQ:FD [2]	Unadjusted [3]	Energy-Adjusted [4]	Deattenuated [5]
	Mean	SD	Mean	SD	*p*-Value [6]	Mean	SD		Pearson	Pearson	Pearson
Grain, Cereal, Bread	4.70	1.67	9.83	2.42	<0.001	5.14	2.30	2.22	0.42	0.33	0.35
Vegetable	1.88	1.13	1.53	0.25	<0.001	−0.38	1.05	0.91	0.16	0.21	0.25
Legumes, Pulses, Seeds	1.15	0.24	1.16	0.14	0.620	0.00	0.26	1.03	0.23	0.34	0.55
Fish, Poultry, Meat, Egg	1.30	0.30	1.74	0.89	<0.001	0.50	0.85	1.41	0.41	0.42	0.51
Milk	1.02	0.11	1.29	0.36	<0.001	0.23	0.29	1.21	0.68	0.66	0.80
Fruits	2.08	2.94	1.66	0.89	0.060	−0.49	2.28	1.13	0.75	0.79	≠
Beverages	1.08	0.28	1.60	1.19	<0.001	0.55	1.05	1.47	0.65	0.85	0.90

* For all correlations, $p < 0.001$. [1] FFQ-FQ represents the difference between intake measured by FFQ and intakes measured by FD; [2] FFQ:FD represents the percent ratio of paired FFQ and FD intake measurements; [3] Calculated based on log-transformed value of average daily consumptions; [4] Calculated based on log-transformed value of average daily consumptions with adjustment for total energy intake using the residual method; [5] Correction were calculated based on energy-adjusted correlation accounting for random within-individual error in the two 3-day FD; [6] Paired *t*-test comparing FFQ and FD in daily average intakes. ≠ Correction coefficient not calculated due to the very large ratio of within-person to between-person variances.

Table 1-4. Degree of association and level of agreement between average daily nutrient intakes by food diary (FD) and by semi-quantitative food questionnaire (FFQ).

Nutrients (Unit)	Average Daily Intake (Unit/Day)					Level of Agreement			Correlation Coefficient between FD and FFQ *		
	FD		FFQ			FFQ-FD [1]		FFQ:FD [2]	Unadjusted [3]	Energy-Adjusted [4]	De-attenuated [5]
	Mean	SD	Mean	SD	*p*-Value [6]	Mean	SD		Pearson	Pearson	Pearson
Protein (g)	44.1	14.6	50.8	13.1	<0.001	6.8	15.5	1.26	0.36	0.51	0.58
Fat (g)	23.0	12.5	21.3	8.7	0.125	−1.7	13.3	1.12	0.38	0.45	0.55
Carbohydrate available (g)	214.6	49.8	326.7	107.8	<0.001	112.1	106.2	1.56	0.25	0.54	0.63
Total dietary fiber (g)	23.7	8.4	26.4	7.6	0.001	2.7	9.6	1.20	0.34	0.45	0.70
Ash (g)	9.2	3.0	10.4	3.0	<0.001	1.2	3.9	1.23	0.19	0.20	0.31
Calcium (mg)	345.5	160.2	299.8	99.1	<0.001	−45.7	167.8	0.98	0.30	0.26	0.34
Iron (mg)	16.1	5.4	21.0	16.7	<0.001	4.9	16.2	1.36	0.38	0.40	0.47
Magnesium (mg)	297.1	92.2	334.8	102.3	<0.001	37.7	117.6	1.20	0.32	0.37	0.68
Phosphorus (mg)	655.1	226.0	873.7	268.9	<0.001	218.7	313.6	1.46	0.30	0.33	0.39
Potassium (mg)	1346.9	463.5	1312.9	386.9	0.438	−33.9	520.5	1.07	0.32	0.45	0.53
Sodium (mg)	939.9	581.9	1033.6	785.4	0.187	93.7	939.1	1.54	0.13	0.17	0.23
Zinc (mg)	6.9	2.3	9.4	3.0	<0.001	2.5	3.3	1.46	0.30	0.36	0.87
Copper (mg)	2.5	0.7	2.7	0.5	0.002	0.2	0.7	1.14	0.21	0.23	0.29
Vitamin A (mcg)	192.0	138.6	334.2	171.1	<0.001	142.2	193.4	2.39	0.21	0.27	0.51
Retinol (mcg)	36.1	45.0	43.5	30.3	0.017	7.4	49.9	3.96	0.17	0.28	0.37
Beta-carotene equivalents (mcg)	2586.6	2594.6	3591.8	1552.5	<0.001	1005.2	2823.7	3.31	0.15	0.20	0.27
Alpha-carotene (mcg)	835.4	656.4	660.3	288.8	<0.001	−175.1	695.0	1.85	0.13	0.22	0.29
Beta-carotene (mcg)	1855.1	1562.4	2972.3	1544.2	<0.001	1117.2	1922.3	2.29	0.17	0.21	0.30
Total cryptoxanthin (mcg)	552.0	400.5	404.5	185.6	<0.001	−147.5	428.2	1.30	0.08	0.22	0.31
Vitamin D (mcg)	2.2	1.2	1.9	0.6	0.002	−0.3	1.2	1.09	0.23	0.45	0.57
Vitamin E (mg)	2.9	1.3	3.3	0.7	<0.001	0.4	1.3	1.32	0.18	0.21	0.26
Thiamin (mg)	1.4	0.3	1.6	0.2	<0.001	0.3	0.3	1.22	0.24	0.33	0.38
Riboflavin (mg)	1.2	0.2	1.4	0.2	<0.001	0.2	0.2	1.22	0.24	0.33	0.38
Niacin equivalents (mg)	6.4	3.3	6.3	3.1	0.761	−0.1	4.0	1.26	0.28	0.30	0.36
Niacin, preformed (mg)	7.8	2.4	11.3	2.6	<0.001	3.5	3.2	1.53	0.26	0.25	0.33
Niacin equivalents from tryptophan (mg)	6.0	3.0	7.3	1.9	<0.001	1.3	3.0	1.48	0.31	0.36	0.41
Vitamin B6 (mg)	1.6	0.4	1.9	0.3	<0.001	0.3	0.4	1.25	0.30	0.38	0.49
Folate (mcg)	140.2	53.1	140.1	40.5	0.984	−0.1	58.4	1.10	0.27	0.26	0.31
L-ascorbic acid (mg)	121.7	68.6	66.4	28.4	<0.001	−55.3	71.1	0.78	0.14	0.14	0.18

* For all correlations, $p < 0.001$. [1] FFQ-FQ represents the difference between intake measured by food frequency questionnaire (FFQ) and intakes measured by FD; [2] FFQ:FD represents the percent ratio of paired FFQ and FD intake measurements; [3] Calculated based on log-transformed value of average daily consumptions; [4] Calculated based on log-transformed value of average daily consumptions with adjustment for total energy intake using the residual method; [5] Correction were calculated based on energy-adjusted correlation accounting for random within-individual error in the two 3-day FD; [6] Paired *t*-test comparing FFQ and FD in daily average intakes.

Table 1-5. Agreement of cross-classification quintiles for food intake assessed by food diary (FD) and by semi-quantitative food questionnaire (FFQ).

Intakes	Same Quintile (%)	Adjacent Quintile (%)	One Quintile Apart (%)	Extreme Quintile (%)	Weighted Kappa (95% CI)
Food Group					
Grain, Cereal, Bread based	26.75	42.00	24.34	6.90	0.20 (0.14, 0.26)
Vegetable based	27.11	37.29	27.23	8.37	0.14 (0.08, 0.20)
Legumes, Pulses, Seeds based	24.40	46.28	25.10	4.24	0.21 (0.15, 0.27)
Fish, Poultry, Meat, Egg based	35.27	46.60	15.17	2.96	0.41 (0.35, 0.46)
Milk based	35.38	30.84	24.95	8.84	0.21 (0.11, 0.30)
Fruits	37.35	32.52	23.80	6.33	0.30 (0.21, 0.40)
Beverages	28.51	36.69	23.60	11.21	0.07 (0.00, 0.14)
Nutrient Intake					
Protein (g)	32.15	45.53	18.89	3.43	0.34 (0.28, 0.41)
Fat (g)	38.77	40.69	18.75	1.80	0.43 (0.38, 0.49)
Carbohydrate available (g)	42.83	33.45	19.48	4.23	0.40 (0.33, 0.47)
Total dietary fiber (g)	33.89	44.63	20.58	0.90	0.42 (0.37, 0.47)
Ash (g)	30.25	37.93	25.32	6.47	0.19 (0.13, 0.25)
Calcium (mg)	29.24	38.46	24.09	8.20	0.14 (0.08, 0.20)
Iron (mg)	27.84	42.85	23.04	6.26	0.22 (0.16, 0.28)
Magnesium (mg)	27.96	42.11	22.78	7.13	0.20 (0.14, 0.26)
Phosphorus (mg)	27.12	38.18	26.12	8.58	0.12 (0.06, 0.18)
Potassium (mg)	28.62	44.39	23.60	3.39	0.29 (0.23, 0.34)
Sodium (mg)	30.42	40.01	20.60	8.97	0.17 (0.10, 0.23)
Zinc (mg)	30.40	39.58	23.76	6.28	0.20 (0.14, 0.27)
Copper (mg)	26.40	40.03	25.07	8.50	0.11 (0.04, 0.17)
Vitamin A (mcg)	29.62	33.46	27.18	9.74	0.09 (0.03, 0.15)
Retinol (mcg)	28.39	38.18	24.46	8.97	0.13 (0.06, 0.20)
Beta-carotene equivalents (mcg)	27.82	39.76	24.43	8.00	0.10 (0.03, 0.16)
Alpha-carotene (mcg)	27.38	44.04	24.87	3.70	0.19 (0.12, 0.25)
Beta-carotene (mcg)	27.32	38.36	25.56	8.76	0.09 (0.02, 0.15)
Total Cryptoxanthin (mcg)	24.30	45.61	23.83	6.27	0.15 (0.09, 0.21)
Vitamin D (mcg)	35.66	38.28	22.42	3.64	0.32 (0.27, 0.37)
Vitamin E (mg)	25.16	40.04	27.03	7.76	0.10 (0.03, 0.16)
Thiamin (mg)	30.94	45.62	21.09	2.34	0.32 (0.26, 0.38)
Riboflavin (mg)	35.24	38.85	21.79	4.11	0.30 (0.24, 0.36)
Niacin equivalents (mg)	23.78	43.50	25.61	7.11	0.13 (0.07, 0.19)
Niacin, preformed (mg)	33.68	38.66	23.46	4.19	0.30 (0.23, 0.36)
Niacin equivalents from tryptophan (mg)	28.63	39.81	25.18	6.40	0.17 (0.11, 0.23)
Vitamin B6 (mg)	26.55	40.23	23.51	9.73	0.10 (0.04, 0.16)
Folate (mcg)	31.57	38.17	23.76	6.49	0.19 (0.13, 0.26)
L-ascorbic acid (mg)	30.96	38.91	21.54	8.58	0.08 (0.04, 0.11)

1.3.5. Integrative Interpretation of Statistical Outcomes

Table 1-6 presents the interpretation and summary of six statistical tests comparing average daily nutrient intakes calculated by FFQ and FD. Summary results showed that total energy intake measured by the FFQ had no evidence of statistically significant proportional bias ($p = 0.04$) and that the FFQ agreed well with the FD in classifying total energy intake, although this agreement may be partly due to chance. FFQ had good validity to capture macronutrient intakes (protein, fat, fiber) at the group level, except for carbohydrates, which had a large percent difference and presence of bias with a significant negative Bland–Altman Spearman's correlation, i.e., the difference became more negative as the mean of the consumption increases. The validity for mineral intakes (calcium, iron, magnesium, phosphorus, potassium, sodium, zinc, and copper) was good at the group level, except for calcium and potassium, which had the presence of proportional bias in the positive direction, i.e., as average intake increases, so does the difference of intake. The supporting individual level validity was acceptable for mineral intakes. Micronutrient intakes had good group and individual level validity for thiamin, riboflavin, niacin, and folate, with no presence of bias. Intake of L-ascorbic acid measured by FFQ had poor validity at both the group and individual levels. Most nutrient intakes measured by FFQ had poor complete agreement with intake measured by FD, suggesting that the FFQ is not suitable for measuring the absolute intakes.

Table 1-6. Statistical test outcomes and interpretations [1] for nutrient intake.

Validation Type	Association	Test of Agreement				Presence, Direction and Extent of Bias
		Complete Agreement	Size and Direction of Error	Including Chances	Excluding Chances	
Statistical Method	Spearman's Correlation [2]	Paired *t*-Test (*p*-Value)	Percent Difference [3] (%)	Cross-Classification (% in Opposite Quintiles)	Weighted Kappa	Bland–Altman Spearman Correlation (*p*-Value)
Intake (unit/day)						
Energy (kcal)	0.35 ^	<0.001	44.0%	9.30	0.14	−0.16 (0.040)
Protein (g)	0.46	<0.001	15.3%	3.43	0.34	0.21 (0.073)
Fat (g)	0.45	<0.001	−7.2%	1.80	0.43	0.24 (0.052)
Carbohydrate available (g)	0.50	<0.001	52.2%	4.23	0.40	−0.32 (<0.001)
Total dietary fiber (g)	0.43	<0.001	11.5%	0.90	0.42	0.08 (0.332)
Ash (g)	0.22	<0.001	12.6%	6.47	0.19	0.04 (0.615)
Calcium (mg)	0.24	<0.001	13.2%	8.20	0.14	0.46 (<0.001)
Iron (mg)	0.32	<0.001	30.3%	6.26	0.22	−0.07 (0.408)
Magnesium (mg)	0.30	<0.001	12.7%	7.13	0.20	0.17 (0.071)
Phosphorus (mg)	0.30	<0.001	33.4%	8.58	0.12	0.01 (0.913)
Potassium (mg)	0.41	0.168	−2.5%	3.39	0.29	0.31 (<0.001)
Sodium (mg)	0.17	<0.001	10.0%	8.97	0.17	−0.15 (0.071)
Zinc (mg)	0.37	<0.001	35.9%	6.28	0.20	0.10 (0.214)
Copper (mg)	0.20	<0.001	8.1%	8.50	0.11	0.15 (0.065)
Vitamin A (mcg)	0.24	<0.001	74.1%	9.74	0.09	0.09 (0.261)
Retinol (mcg)	0.26	0.686	20.5%	8.97	0.13	0.07 (0.374)
Beta–carotene equivalents (mcg)	0.19	<0.001	38.9%	8.00	0.10	0.26 (0.081)
Alpha-carotene (mcg)	0.19	<0.001	−21.0%	3.70	0.19	0.41 (<0.001)
Beta-carotene (mcg)	0.15	<0.001	60.2%	8.76	0.09	0.22 (0.001)
Total Cryptoxanthin (mcg)	0.21	<0.001	−26.7%	6.27	0.15	0.36 (<0.001)
Vitamin D (mcg)	0.41	<0.001	−13.5%	3.64	0.32	0.52 (0.293)
Vitamin E (mg)	0.21	<0.001	15.1%	7.76	0.10	0.32 (0.310)
Thiamin (mg)	0.28	<0.001	18.4%	2.34	0.32	0.08 (0.293)
Riboflavin (mg)	0.24	<0.001	19.0%	4.11	0.30	0.29 (0.002)
Niacin equivalents (mg)	0.22	0.463	−0.8%	7.11	0.13	0.44 (<0.001)
Niacin, preformed (mg)	0.25	<0.001	44.2%	4.19	0.30	−0.15 (0.051)
Niacin equivalents from tryptophan (mg)	0.24	<0.001	22.3%	6.40	0.17	0.29 (0.082)
Vitamin B6 (mg)	0.30	<0.001	20.9%	9.73	0.10	0.20 (0.051)
Folate (mcg)	0.25	0.613	−0.1%	6.49	0.19	0.15 (0.063)
L-ascorbic acid (mg)	0.17	<0.001	−45.4%	8.58	0.08	0.66 (<0.001)

[1] Interpretation criteria for statistical tests (good in orange, acceptable in purple, poor in red); [2] Energy adjusted Spearman's correlation between average daily nutrient intakes calculated from FFQ and FD (all *p*-values < 0.001); [3] (FFQ-FD)/FD × 100%. ^ Unadjusted Spearman's correlation between average daily energy intakes calculated from FFQ and FD (*p*-values < 0.001).

1.4. Discussion

We developed a dish-based semi-quantitative FFQ consisting of 42 food items to assess long-term dietary habits of participants in a longitudinal arsenic biomonitoring study in rural Bangladesh. Performance of the FFQ was evaluated by comparing average food intake and nutrient intakes derived from this instrument with those recorded in two 3-day FD using a combination of six statistical tests. Total energy intake measured by the two 3-day FD had good-to-strong correlations ($r > 0.7$), and the same was true for all macronutrient intakes, dietary fiber, and some micronutrients, including potassium, retinol, vitamin D, niacin, vitamin B6, and folate, after accounting for within-person variations. Caution must be exercised when using the FFQ to assess populations with wide ranges of carbohydrate and potassium intakes since proportional bias existed at the group level. The validity of the FFQ in assessing intake of vitamin A, L-asorbic acid, and total cryptoxanthin was questionable, indicated by weak association, a low level of agreement, and the presences of bias between the FFQ and the two 3-day FD. The FFQ was also not suitable to measure absolute intake.

Compared with previously validated FFQ in Bangladesh that used the USDA nutrient database [9], our FFQ had stronger deattenuated correlation for total energy, protein, carbohydrate, dietary fiber, potassium, iron, magnesium, thiamin, copper, zinc, calcium, folate, retinol, vitamin C, and vitamin E. The use of the Food Composition Table in Bangladesh also allowed us to assess the intake of additional micronutrients that were not available in the previous FFQ, including alpha-carotene, beta-carotene, total cryptoxanthin, and vitamin D. We did not observe the problem of under-reporting of meat and over-reporting of fruits that was observed in the previous food-based FFQ used in Bangladesh [9] and other Westernized countries [40–43]. The level of overestimation and underestimation of food consumption

measured by the FFQ, as compared to those recorded in the FD, was also much lower. Contrastingly, our FFQ overestimated the consumption of grain, cereal, and bread-based dishes, which were not observed in the previous food-based FFQ in Bangladesh. Our dish-based FFQ included a dish of homemade snacks, of which the main ingredient is flour. Consumption of homemade snacks may be considered a symbol of better economic status in rural Bangladesh; thus, its consumption may be over-reported. Since the previous food-based FFQ did not included snacks, it did not observe this problem.

The seasonal variation in nutrient intake of our study population during the study period was small for total energy, protein, and carbohydrate intakes. Larger seasonal variations were found for vitamin D, beta-carotene, and vitamin A intakes. Small seasonal variation for total energy was expected since energy intake and expenditure is sensitivity-regulated according to body size and metabolism level. Protein and carbohydrates are the main contributors to daily energy intake and are distributed across different types of foods and thus expected to have small seasonal variations. Micronutrients, on the contrary, tend to concentrate in specific foods, so the level of micronutrient intakes is highly dependent on food choices and food availability [10]. Previous studies in Bangladesh showed evidence of striking seasonal variations in vitamin A consumption 44–46], and a similar observation was found based on our result of the FD.

In settings with wide seasonal variation in diet, the ability to assess the validity of a FFQ in measuring long-term intakes of micronutrients is usually limited and the number of days required for dietary monitoring is greater, in order to allow for true estimation of intakes [47]. A previous study had found poor validity associated with food and nutrient intakes that had large seasonal variations [44]. The utilization of the dish-based approach in our study reduced the severity of this problem. Instead of asking the consumption of a long list of fruits and vegetables,

whose availability varied largely with seasons, our strategy was to ask the consumption of vegetable- and fruit-based dishes in the FFQ, i.e., leafy vegetables (sak), fried vegetable (bhaji), mixed vegetables (labra), mashed vegetables (bhorta), vegetable curry (torkarir jhole), dal with vegetables, meat with grains, legumes, vegetables (dhansak), fruit, mashed fruit (bhorta), and fruit pickle (aachar). By asking about the consumption of food dishes, we reduced the variability in consumption levels caused by the availability of certain food ingredients. We are also confident that the average of the mixed dish recipes collected in different seasons from 20 households was representative of the nutrient composition for the food dishes.

Our study has several other strengths. All of our FFQs were obtained by trained interviews via in-person, face-to-face interviews. Interviewers can help collect more detailed data; they encourage participation, and can reduce missing data on the dietary assessment. Only 0.4% of all FFQ questions had a missing response. Compared to self-administered FFQ, where food items may be left blank for various reasons, i.e., because the food was not consumed, because of difficulties remembering the frequency or amount of intake, or due to an oversight [48], having interviewers enabled us to clarify the reason for missing and provided more accurate assessment of dietary intake. This advantage is particularly important in areas, such as Bangladesh, where rural people are not closely familiar with epidemiological studies and have low literacy levels, where having an interviewer is crucial and important. All of the dishes recorded in the FD were available in the FFQ, suggested our FFQ had good ability in capturing the range of food consumed by the study population.

The main limitation of the study was that the FD was only collected from the female head of the household who was in charge of meal preparation, but not for all participants. In Bangladesh, it is common for the female head of the households to be in charge of cooking meals

for the entire family at home and for all family members to eat meals cooked at home. Therefore, we found that it was appropriate to estimate the FD for the other members of the household using the AME fraction. Successfully implementation of the AME depends on accurate estimate of energy requirement and ensuring that the consumption of all food is proportional to one's energy requirement. We used energy requirements derived from a national household survey in Bangladesh, which carefully calculated caloric requirements based on body weight, basal metabolic rate, and physical activity levels for sex and age groups [20]. Although the AME method had not worked consistently well across nations, studies had found good applicability in Bangladesh [49,50]. Sensitivity analysis among the 47 female heads of household supported our use of the AME method, in that it increased the sample size and gave higher statistical power to provide disaggregated results for different age and sex groups.

Another limitation was that the number of FD days collected in the study was only six days due to limited resources and manpower. The lack of sufficient days of FD collection might weaken the statistical power to capture the dietary variation of some micronutrients with high variance ratios [51]. Nevertheless, our study found that the dietary data captured by the two 3-day FD did have higher than 80% power to accurately classify the population into tertiles with 95% confidence for total energy, protein, fat, carbohydrate, folate, niacin, riboflavin, iron, thiamin, potassium, phosphorus, and vitamin E [52].

1.5. Conclusions

The dish-based semi-quantitative FFQ developed to assess long-term habitual diet for rural Bangladeshi population provided valid estimates on relative intakes of food dishes and nutrients both at the group and the individual levels for total energy, all macronutrients, and some micronutrients, such as folate, retinol, thiamin, and riboflavin. The instrument had enough

statistical power to accurately rank and classify dietary intake into at least three levels. The dish-based FFQ had low participant burden and generated data that was easy to analyze, and is a useful tool to assess dietary intake in large epidemiology studies.

1.6. References

1. Ahmed, A.U. *Patterns of Food Consumption and Nutrition in Rural Bangladesh*; International Food Policy Research Institute (IFPRI): Washington, DC, USA, 1993.
2. Hassan, N.; Ahmad, K. Intra-familial distribution of food in rural Bangladesh. *Food Nutr. Bull.* **1984**, *5*, 90.
3. Karim, M.R.; Ahmad, S.A. Nutritional Status among the Children of Age Group 5–14 Years in Selected Arsenic Exposed and Non-Exposed Areas of Bangladesh. *J. Fam. Reprod. Health* **2014**, *8*, 161.
4. World Bank. *Bangladesh—Household Income and Expenditure Survey: Key Findings and Results 2010*; The World Bank Group: Washington, DC, USA, 2011.
5. Akhtaruzzaman, M.; Khan, N.I.; Islam, S.N. *Nutrition, Health and Demographic Survey of Bangladesh-2011*; United States Agency for International Development (USAID) Bangladesh: the Dhaka, Bangladesh 2013.
6. Ahmed, A. *Bangladesh Integrated Household Survey (BIHS) 2011–2012*; International Food Policy Research Institute (IFPRI): Washington, DC, USA, 2013.
7. Helen Keller International (HKI) and James P. Grant School of Public Health (JPGSPH). *States of food security and nutrition in Bangladesh: 2013;* HKI and JPGSPH: Dhaka, Bangladesh, 2014.
8. Willet, W. Food Frequency Methods. In *Nutritional Epidemiology*; Oxford University Press: Oxford, UK, 2013; pp. 71–72.
9. Chen, Y.; Ahsan, H.; Parvez, F.; Howe, G.R. Validity of a food-frequency questionnaire for a large prospective cohort study in Bangladesh. *Br. J. Nutr.* **2004**, *92*, 851–859.
10. Willett, W.C. *Nutritional Epidemiology*, 2nd ed.; Willett, W.C., Ed.; Oxford University

Press: New York, NY, USA, 1998.

11. Cade, J.E.; Burley, V.J.; Warm, D.L.; Thompson, R.L.; Margetts, B.M. Food-frequency questionnaires: A review of their design, validation and utilisation. *Nutr. Res. Rev.* **2004**, *17*, 5–22.
12. Stark, A. An historical review of the Harvard and the National Cancer Institute food frequency questionnaires: Their similarities, differences, and their limitations in assessment of food intake. *Ecol. Food Nutr.* **2002**, *41*, 35–74.
13. Kile, M.L.; Houseman, E.A.; Breton, C.V.; Smith, T.; Quamruzzaman, Q.; Rahman, M.; Mahiuddin, G.; Christiani, D.C. Dietary arsenic exposure in Bangladesh. *Environ. Health Perspect.* **2007**, *115*, 889–893.
14. Kile, M.L.; Houseman, E.A.; Breton, C.V.; Quamruzzaman, Q.; Rahman, M.; Mahiuddin, G.; Christiani, D.C. Association between total ingested arsenic and toenail arsenic concentrations. *J. Environ. Sci. Health A Toxic Hazard. Subst. Environ. Eng.* **2007**, *42*, 1827–1834.
15. Shaheen, N.; Rahim, A.T.;Mohiduzzaman, M; Banu, C.P; , Bari, L.; Tukun, B.; Mannan, M.; Bhattacharjee, L.; Stadlmayr, B. *Food Composition Table for Bangladesh*; Institute of Nutrition and Food Science; Centre for Advanced Research in Sciences; University of Dhaka: Dhaka, Bangladesh, 2013.
16. Cade, J.; Thompson, R.; Burley, V.; Warm, D. Development, validation and utilisation of food-frequency questionnaires—A review. *Public Health Nutr.* **2002**, *5*, 567–587.
17. Lombard, M.J.; Steyn, N.P.; Charlton, K.E.; Senekal, M. Application and interpretation of multiple statistical tests to evaluate validity of dietary intake assessment methods. *Nutr. J.* **2015**, *14*, 40.

18. Kile, M.L.; Houseman, E.A.; Rodrigues, E.; Smith, T.J.; Quamruzzaman, Q.; Rahman, M.; Mahiuddin, G.; Su, L.; Christiani, D.C. Toenail arsenic concentrations, *GSTT1* gene polymorphisms, and arsenic exposure from drinking water. *Cancer Epidemiol. Biomark. Prev.* **2005**, *14*, 2419–2426.
19. Weisell, R.; Dop, M.C. The adult male equivalent concept and its application to Household Consumption and Expenditures Surveys (HCES). *Food Nutr. Bull.* **2012**, *33*, S157–S162.
20. Nahar, Q. *Desirable Dietary Pattern for Bangladesh*; National Food Policy Capacity Strengthening Programme: Dhaka, Bangladesh, 2013.
21. Bermudez, O.I.; Lividini, K.; Smitz, M.-F.; Fiedler, J.L. Estimating micronutrient intakes from Household Consumption and Expenditures Surveys (HCES): An example from Bangladesh. *Food Nutr. Bull.* **2012**, *33*, S208–S213.
22. Charrondiere, R.U.; Stadlmayr, B.; Haytowitz, D.; Oseredczuk, M.; Ireland, J.; Wolmarans, P.; Rittenschober, D.; Selly, B.; Puwastien, P.; Reykdal, O.; et al. *FAO/INFOODs Guidelines for Checking Food Composition Data Prior to the Publication of a User Table/Database Version 1.0*; International Network of Food Data Systems (INFOODS): Rome, Italy, 2012.
23. De Onis, M.; Onyango, A.W.; Borghi, E.; Siyam, A.; Nishida, C.; Siekmann, J. Development of a WHO growth reference for school-aged children and adolescents. *Bull. World Health Organ.* **2007**, *85*, 660–667.
24. Shrout, P.E.; Fleiss, J.L. Intraclass correlations: Uses in assessing rater reliability. *Psychol. Bull.* **1979**, *86*, 420–428.
25. Sampat, M.P.; Whitman, G.J.; Stephens, T.W.; Broemeling, L.D.; Heger, N.A.; Bovik, A.C.; Markey, M.K. The reliability of measuring physical characteristics of spiculated masses on

mammography. *Br. J. Radiol.* **2006**, *79*, S134–S140.

26. Winer, B. *Statistical Principles in Experimental Design*; McGraw-Hill Book Company: New York, NY, USA, 1962.
27. Black, A.E.; Cole, T.J.; Wiles, S.J.; White, F. Daily variation in food intake of infants from 2 to 18 months. *Hum. Nutr. Appl. Nutr.* **1983**, *37*, 448–458.
28. Kuehneman, T.; Stanek, K.; Eskridge, K.; Angle, C. Comparability of four methods for estimating portion sizes during a food frequency interview with caregivers of young children. *J. Am. Diet. Assoc.* **1994**, *94*, 548–551.
29. Venter, C.S.; MacIntyre, U.E.; Vorster, H.H. The development and testing of a food portion photograph book for use in an African population. *J. Hum. Nutr. Diet.* **2000**, *13*, 205–218.
30. Willett, W.; Stampfer, M.J. Total energy intake: Implications for epidemiologic analyses. *Am. J. Epidemiol.* **1986**, *124*, 17–27.
31. Beaton, G.H.; Milner, J.; McGuire, V.; Feather, T.E.; Little, J.A. Source of variance in 24-hour dietary recall data: Implications for nutrition study design and interpretation. Carbohydrate sources, vitamins, and minerals. *Am. J. Clin. Nutr.* **1983**, *37*, 986–995.
32. Gibson, R.S. *Principles of Nutritional Assessment*; Oxford University Press: Oxford, UK 2005.
33. Cicchetti, D.V.; Allison, T. A new procedure for assessing reliability of scoring EEG sleep recordings. *Am. J. EEG Technol.* **1971**, *11*, 101–109.
34. Feinstein, A.R.; Cicchetti, D. V High agreement but low kappa: I. The problems of two paradoxes. *J. Clin. Epidemiol.* **1990**, *43*, 543–549.
35. Dehghan, M.; Martinez, S.; Zhang, X.; Seron, P.; Lanas, F.; Islam, S.; Merchant, A.T. Relative validity of an FFQ to estimate daily food and nutrient intakes for Chilean adults.

Public Health Nutr. **2013**, *16*, 1782–1788.

36. Bland, J.M.; Altman, D.G. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet* **1986**, *1*, 307–310.
37. Lee, R.; Neiman, D. *Nutritional Assessment, the* 6th ed.; McGraw-Hill Education: New York City, USA 2012.
38. MacIntyre, U.E.; Venter, C.S.; Vorster, H.H. A culture-sensitive quantitative food frequency questionnaire used in an African population: 2. Relative validation by 7-day weighted records and biomarkers. *Public Health Nutr.* **2001**, *4*, 63–71.
39. Costa, M.D.M.; Takeyama, L.; Voci, S.M.; Slater, B.; Silva, M.V. Within- and between-person variations as determinant factors to calculate the number of observations to estimate usual dietary intake of adolescents. *Rev. Bras. Epidemiol.* **2008**, *11*, 541–548.
40. Salvini, S.; Hunter, D.J.; Sampson, L.; Stampfer, M.J.; Colditz, G.A.; Rosner, B.; Willett, W.C. Food-based validation of a dietary questionnaire: The effects of week-to-week variation in food consumption. *Int. J. Epidemiol.* **1989**, *18*, 858–867.
41. Feskanich, D.; Rimm, E.; Giovannucci, E.; Colditz, G.; Stampfer, M.; Litin, L.; Willett, W. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. *J. Am. Diet. Assoc.* **1993**, *93*, 790–796.
42. Macedo-Ojeda, G.; Vizmanos-Lamotte, B.; Márquez-Sandoval, Y.F.; Rodríguez-Rocha, N.P.; López-Uriarte, P.J.; Fernández-Ballart, J.D. Validation of a semi-quantitative food frequency questionnaire to assess food groups and nutrient intake. *Nutr. Hosp.* **2013**, *28*, 2212–2220.
43. Marventano, S.; Mistretta, A.; Platania, A.; Galvano, F.; Grosso, G. Reliability and relative validity of a food frequency questionnaire for Italian adults living in Sicily, Southern Italy.

Int. J. Food Sci. Nutr. **2016**, *67*, 857–864.

44. Abdullah, M.; Wheeler, E.F. Seasonal variations, and the intra-household distribution of food in a Bangladeshi village. *Am. J. Clin. Nutr.* **1985**, *41*, 1305–1313.
45. Brown, K.H.; Black, R.E.; Robertson, A.D.; Becker, S. Effects of season and illness on the dietary intake of weanlings during longitudinal studies in rural Bangladesh. *Am. J. Clin. Nutr.* **1985**, *41*, 343–355.
46. Abdullah, M.; Ahmed, L. Validating a simplified approach to the dietary assessment of vitamin A intake in preschool children. *Eur. J. Clin. Nutr.* **1993**, *47*, 115–122.
47. Boyle, A. *Community Nutrition in Action: An Entrepreneurial Approach*, 6th Ed.; Cengage Learning: Belmont, OH, USA, 2012.
48. Michels, K.B.; Willett, W.C. Self-administered semiquantitative food frequency questionnaires: Patterns, predictors, and interpretation of omitted items. *Epidemiology* **2009**, *20*, 295–301.
49. Berti, P.R. Intrahousehold distribution of food: A review of the literature and discussion of the implications for food fortification programs. *Food Nutr. Bull.* **2012**, *33*, S163–S169.
50. Sununtnasuk, C. *Individual Energy and Nutrient Intake from a 24-Hour and 7-Day Recall: Comparing Estimates Using the 2011/2012 Bangladesh Integrated Household Survey*; United States Agency for International Development (USAID): Washington, DC, USA, 2014.
51. Cole, T. *Design Concepts in Nutritional Epidemiology*; Margetts, B., Nelson, M., Eds.; Oxford Scholarship Online: Oxford, UK, 1991.
52. Karkeck, J.M. Improving the use of dietary survey methodology. *J. Am. Diet. Assoc.* **1987**, *87*, 869–871.

1.7. Supplementary Material

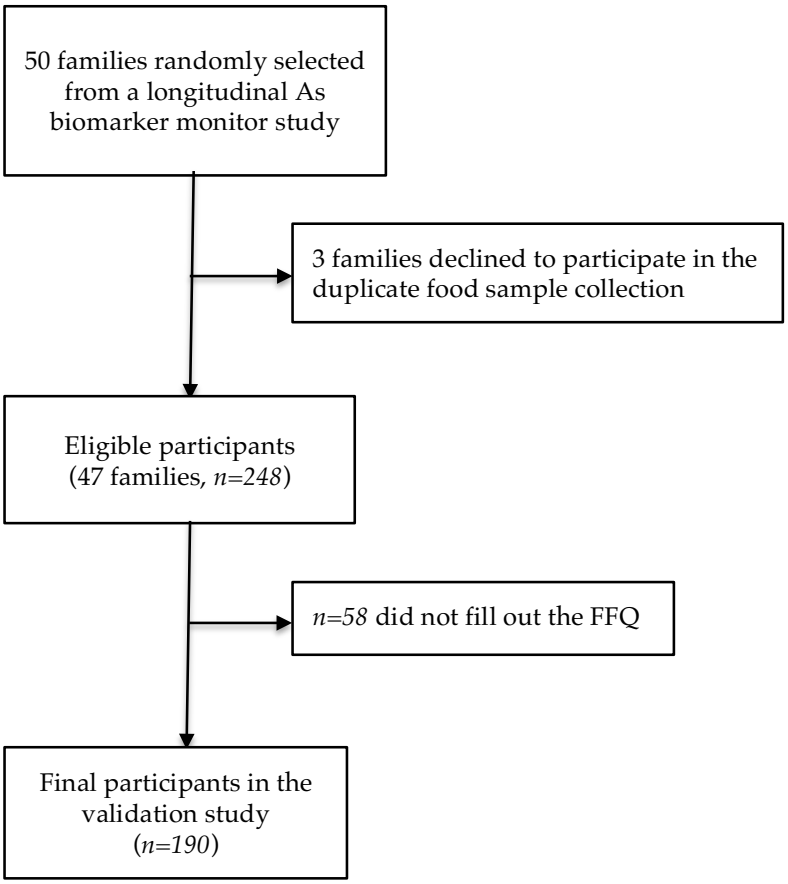

Figure S1-1. Flowchart of the study participants

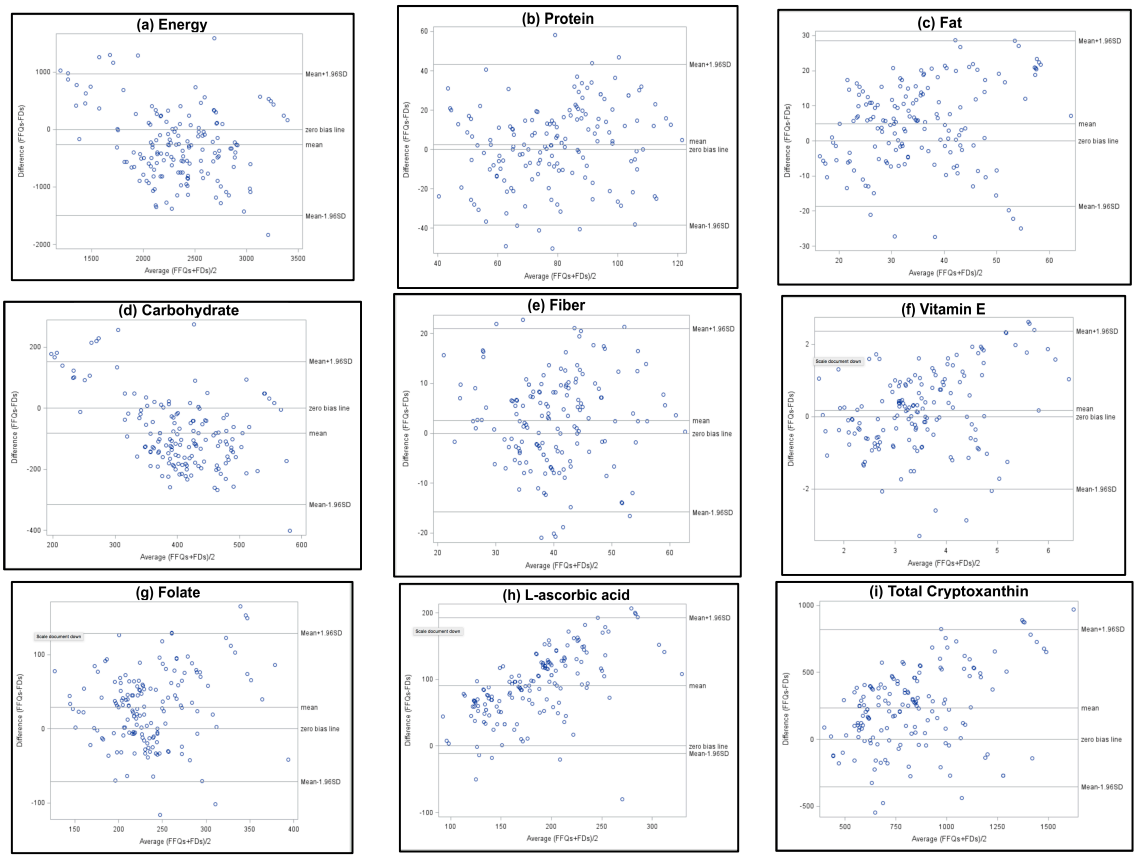

Figure S1-2. Bland–Altman plots for selected nutrient intakes showing agreement between paired means and differences in nutrient intakes measured by FFQ and FD. (**a**) Total energy (kcal/day); (**b**) Protein (g/day); (**c**) fat (g/day); (**d**) carbohydrate available (g/day); (**e**) total dietary fiber (g/day); (**f**) vitamin E (mg/g); (**g**) folate (mcg/day); (**h**) L-ascorbic acid (mg/day); (**i**) total cryptoxanthin (mcg/day).

Table S1-1. Food dishes in each of food groups in the semi-quantitative dish-based Food Frequency Questionnaire (FFQ).

Food Group	Food Dishes: English Name (Local Name)
Grain, Cereal, Bread based	Plain rice (Bhaat, Panta bhaat) Special rice (Khichuri, Pulao, Biriyani) Rice cereal (Chira, Muri, Khoi, Murki) Plain bread (Atta ruti, Pau ruti) Fried bread (Porota, Luchi) Home made snacks (Pitha-puli)
Vegetable based	Leafy vegetable (Sak) Mashed vegetable (Bhorta) Fried vegetable (Bhaji) Mixed vegetable (Labra) Vegetable Curry (Torkarir jhole)
Legumes, Pulses, Seeds based	Plain dal Dal with vegetables
Fish, Poultry, Meat, Egg based	Fish Fry (Mach bhaji) Fish curry (Mach er jhole) Fish curry with vegetable Fish head with dal or vegetables Fish egg fry (Maccher dim bhaji) Dried fish with vegetable Meat curry with potato Meat with legumes (Halim) Meat with, grains, legumes, vegetables (Dhansak) Meat kabab Egg curry (Dim er jhole)
Milk based	Plain milk (Doodh) Cottage cheese (chana) Yogurt (Doi) Yogurt drink (Ghole, Matha, Borhani) Thickened milk (Khoa, kheer) Rice pudding (Payesh) Vermicelli (Semai) Sweetmeats (Mishti)
Fruits	Fruit Mashed fruit (Bhorta) Fruit pickle (Aachar)
Beverages	Plain water Fruit juice Soft drinks Tea Coffee

Table S1-2. Socio-demographic characteristics of the 47 female heads of household.

	n	**%**
All participants	47	
Sex		
Male	0	0.0%
Female	47	100.0%
Age		0.0%
20–30	12	25.5%
31–40	22	46.8%
41–50	11	23.4%
51–65	2	4.3%
>65	0	0.0%
BMI		
<18.5	7	14.9%
18.5–24.9	29	61.7%
25.0+	11	23.4%
Education		
Illiterate	6	12.8%
Able to write	24	51.1%
Primary Education	6	12.8%
Secondary Education	9	19.1%
Higher Secondary Education	2	4.3%
Job Type		
Factory Labor	0	0.0%
Businessman	1	2.1%
Housewife	45	95.7%
Student	1	2.1%

Table S1-3. Degree of association and level of agreement between average daily food group intakes by FD and FFQ reported by the 47 female heads of households.

Food Group (Subgroup)	Average Daily Consumption (Serving/Day)				Correlation Coefficient between FD and FFQ		
	FD (n = 47)		FFQ (n = 47)		Unadjusted [1]	Energy-Adjusted [1]	Corrected [3]
	Mean	SD	Mean	SD	Pearson	Pearson	Pearson
Grain, Cereal, Bread based	4.99	1.64	8.75	2.32	0.40	0.29	0.50
(Rice)	4.13	1.21	6.47	1.63	0.23	0.34	0.41
(Bread)	0.77	1.54	2.25	2.38	0.45	0.62	0.72
Vegetable based	1.14	1.78	0.56	0.29	0.15	0.15	0.36
(Leafy Vegetable)	0.03	0.10	0.11	0.10	0.23 $^{\approx}$	0.43	0.47
(Other Vegetable)	1.11	1.78	0.44	0.25	0.13	0.29	0.41
Legumes, Pulses, Seeds based	0.19	0.30	0.18	0.14	0.41	0.16	0.43
Fish, Poultry, Meat, Egg based	0.52	0.33	0.60	0.65	0.13	0.20	0.42
(Meat)	0.05	0.13	0.10	0.20	0.61	0.25	0.64
(Fish)	0.25	0.31	0.42	0.60	0.20	0.38	0.52
(Eggs)	0.08	0.21	0.07	0.07	0.16	0.56	0.68
Milk based	0.03	0.14	0.68	0.65	0.47	0.64	0.68
Fruits	1.57	4.24	0.91	0.94	0.50	0.35	0.71
Beverages	0.09	0.32	1.04	2.20	0.40	0.76	0.80

$^{\approx}$ $p > 0.1$, for all other correlation, $p < 0.05$. [1] Calculated based on log-transformed value of average daily consumptions. [2] Calculated based on log-transformed value of average daily consumptions with adjustment for total energy intake using the residual method. [3] Correction were calculated based on energy-adjusted correlation according for random within-individual error in the two 3-day FD.

Table S1-4. Degree of association and level of agreement between average daily nutrients intakes by FD and by FFQ reported by the 47 female heads of households.

	Average Daily Dietary Intake (Unit/Day)							Correlation Coefficient between FD and FFQ		
	FD		FFQ		Difference (FFQ-FD) [1]		FD:FFQ	Unadjusted [2]	Energy-Adjusted [3]	Corrected [4]
Nutrients (Unit)	Mean	STD	Mean	STD	Mean	STD	%	Pearson *r*	Pearson *r*	Pearson *r*
Protein (g)	46.77	1.32	51.24	1.46	2.99	16.23	109.56%	0.33	0.41	0.61
Fat (g)	17.80	1.50	22.10	1.78	7.36	14.37	124.16%	0.28	0.48	0.93
Carbohydrate available (g)	287.34	1.87	236.46	1.29	−72.74	97.48	82.29%	0.33	0.39	0.70
Total dietary fiber (g)	24.05	1.38	26.80	1.39	2.16	9.90	111.43%	0.33	0.35	0.72
Ash (g)	9.89	1.33	10.69	1.34	1.07	3.97	108.09%	0.11 $^{\infty}$	0.20	0.41
Calcium (mg)	290.39	1.35	386.78	1.42	109.10	171.87	133.19%	0.18	0.23	0.38
Iron (mg)	19.83	1.51	19.03	1.59	−0.08	8.55	95.97%	0.28	0.18	0.25
Magnesium (mg)	322.43	1.38	351.37	1.38	24.17	112.14	108.98%	0.24	0.18	0.29
Phosphorus (mg)	834.69	1.45	765.01	1.38	−77.30	311.32	91.65%	0.14	0.15	0.23
Potassium (mg)	1252.93	1.33	1554.75	1.38	347.08	571.33	124.09%	0.18	0.16	0.23
Sodium (mg)	715.22	1.95	815.95	2.10	102.69	981.81	114.08%	0.19	0.21	0.41
Zinc (mg)	9.03	1.35	8.00	1.33	−0.87	2.94	88.59%	0.21	0.18	0.42
Copper (mg)	2.66	1.19	2.92	1.23	0.30	0.73	109.77%	0.13	0.15	0.23
Vitamin A (mcg)	290.67	1.43	184.00	1.82	−103.42	201.88	63.30%	0.13	0.31	- $^{\neq}$
Retinol (mcg)	23.81	2.33	20.75	3.33	3.18	52.79	87.15%	0.22	0.21	0.39
Beta-carotene equivalents (mcg)	3173.82	1.54	2022.65	2.52	−639.16	3358.32	63.73%	0.16	0.22	0.36
Alpha-carotene (mcg)	544.28	1.70	680.19	2.56	284.62	762.82	124.97%	0.21	0.28	0.47
Beta-carotene (mcg)	2776.81	1.46	1694.72	2.20	−864.53	2083.81	61.03%	0.14	0.25	0.40
Total Cryptoxanthin (mcg)	358.42	1.66	493.50	2.19	230.04	476.18	137.69%	0.09 $^{\approx}$	0.22	0.39
Vitamin D (mcg)	1.83	1.25	2.01	1.77	0.84	1.32	109.84%	0.10 $^{\approx}$	0.31	0.48
Vitamin E (mg)	3.16	1.22	3.15	1.45	0.23	1.40	99.68%	0.13	0.15	0.25
Thiamin (mg)	1.61	1.11	1.63	1.13	0.03	0.24	101.24%	0.21	0.12 $^{\infty}$	0.19
Riboflavin (mg)	1.43	1.10	1.45	1.14	0.04	0.21	101.40%	0.20	0.18	0.28
Niacin equivalents (mg)	5.90	1.42	6.82	1.71	1.73	4.15	115.59%	0.15	0.15	0.21
Niacin, preformed (mg)	10.49	1.41	9.06	1.27	−1.91	3.10	86.37%	0.23	0.15	0.21
Niacin equivalents from tryptophan (mg)	7.10	1.30	6.67	1.57	0.02	3.41	93.94%	0.21	0.25	0.33
Vitamin B6 (mg)	1.89	1.15	1.89	1.19	0.04	0.38	100.00%	0.19	0.20	0.29
Folate (mcg)	130.06	1.34	156.82	1.40	24.96	62.05	120.58%	0.23	0.24	0.34
L-ascorbic acid (mg)	60.18	1.51	122.29	1.79	72.26	77.03	203.21%	0.14	0.16	0.27

$^{\approx}$ $p > 0.1$, $^{\infty}$ $0.05 \leq p < 0.1$, for all other correlation, $p < 0.05$. [1] Difference between intake measured by FFQ and intake measured by FD. [2] Correlations were calculated based on log-transformed value of average daily consumptions. [3] Correlations were calculated based on log-transformed value of average daily consumptions with adjustment for total energy intake using the residual method. [4] Correction were calculated based on energy-adjusted correlation according for random within-individual error in the two 3-day FD. $^{\neq}$ Correction coefficient not calculated due to very large ratio of within-person to between-person variances.

Chapter 2. Associations between Diet and Toenail Arsenic Concentration among Pregnant Women in Bangladesh: A Prospective Study

Abstract: This prospective study evaluated the relationship between long-term dietary habits and total arsenic (As) concentration in toenail clippings in a cohort of 1616 pregnant women in the Bangladeshi administrative regions of Sirajdikhan and Pabna Sadar. Diet was assessed at Gestation Week 28 and at Postpartum Month 1, using a locally-validated dish-based semi-quantitative food-frequency questionnaire. Toenail As concentration was analyzed by microwave-assisted acid digestion and inductively coupled plasma mass spectrometry. Associations between natural log-transformed consumption of individual food items and temporally matched natural log-transformed toenail As concentration were quantified using general linear models that accounted for As concentration in the primary drinking water source and other potential confounders. The analysis was stratified by As in drinking water (≤50 μg/L versus >50 μg/L) and the time of dietary assessment (Gestation Week 28 versus Postpartum Week 1). Interestingly, toenail As was not significantly associated with consumption of plain rice as hypothesized. However, toenail As was positively associated with consumption of several vegetable, fish and meat items and was negatively associated with consumption of rice, cereal, fruits, and milk based food items. Further studies in pregnant women are needed to compare As metabolism at different levels of As exposure and the interaction between dietary composition and As absorption.

Keywords: food frequency questionnaire; arsenic exposure; pregnancy; Bangladesh; dietary assessment

2.1. Introduction

The World Health Organization includes arsenic (As) in its list of 10 chemicals of major public health concern. The many adverse health outcomes linked to As exposure include skin lesions, cancers, and cardiovascular diseases [1]. High concentrations of this human toxicant in ground water have been reported in Argentina, Bangladesh, Chile, China, India, Mexico, and the United States of America. Therefore, As in drinking water is a global health concern that may impact over 200 million people [2,3]. In areas with high water As levels in the ground water, As exposure may occur directly through consumption of As-contaminated drinking water or indirectly through consumption of foods, e.g., agricultural produce and livestock that accumulate As from contaminated water, soil, pesticides, feed, feed supplements, and foraged grasses and plants [4–9]. In areas without As contamination, seafood is the main dietary source of arsenic [10]. Arsenic exists in organic and inorganic forms, both of which are readily absorbed (70%–90%) by the gastrointestinal tract. Inorganic forms of arsenic, including the trivalent arsenite (As^{III}) and the pentavalent arsenate (As^{V}), are more toxic compared to the organic forms, such as arsenobetaine, arsenolipids, and arsenosugars that are mainly found in fish and shellfish [11]. Detailed exposure assessments show that As in food and As in drinking water contribute similarly to the internal dose of As intake [12]. Intake of As from food had often been overlooked due to the complexity of assessing As exposure from diet. Nevertheless, total As ingested by an individual may be underestimated if only drinking water As concentration is considered since As accumulates in food during cultivation and during cooking, especially in areas with high As concentrations in the ground water [9].

In Bangladesh, which is an area of endemic high inorganic As (iAs) exposure, accurately estimating dietary exposure to As is particularly important because the iAs concentration in

drinking water typically exceeds the standard of 50 μg/L (national drinking water standard for iAs in Bangladesh) [13]. In the USA and in Europe, the adverse health effects of chronic dietary exposure [9] to iAs are well documented. However, the USA and Europe have only recently recognized the need for regulatory limits on As due to a lack of data for the effects of As ingestion from food and due to the difficulty of assessing As exposure risk [9]. To fill this gap in the literature, we aimed to investigate the relationship between diet and total As exposure.

Arsenic in cooked rice has been studied extensively. The As level in rice varies with the environmental conditions during cultivation [14–16] and with the method and water used for cooking [14,17–19]. Elevated As species in urine have been associated with high consumption of rice and rice-based products [20–22]. A survey of lifestyle and dietary habits in a Japanese population showed that higher than average consumption of rice (four or more bowls per day) was associated with a significantly higher average concentration of toenail As [23]. Particularly high As concentrations have been reported not only in cereals and cereal-based products [24–28], but also in fish and seafood [24–26,29,30] and vegetables [31–33]. While the majority of the As detected in cereal, grains, vegetables, and spices were iAs, fish and seafood contained mostly the organic forms of As and only a small portion of iAs [10].

Arsenic is a potential reproductive toxin since iAs and its methylated metabolites can readily cross the placenta [34,35]. In vivo studies show that even low levels of As exposure can induce oxidative stress [36], DNA methylation, altered gene expression, and free radical formation [37]. A systematic review of population-based studies found that high levels of groundwater iAs (≥50 μg/L) were associated with significantly increased risk of spontaneous abortion and stillbirth, and moderately increased risk of neonatal and infant mortality [38]. The most recent studies indicate that even low levels of iAs exposure in pregnant women are

associated with lower than normal weight, length, chest circumference, and head circumference in newborns [38–41]. Thus, pregnant women are particularly vulnerable to adverse health effects of dietary As exposure [42]. However, the relationship between dietary patterns and As exposure has not been examined specifically in pregnant women. As part of our ongoing studies of the associations between As exposure and maternal and child health, this prospective study evaluated the association between long term diet and total As concentration in toenail clippings in a cohort of pregnant women in Bangladesh.

2.2. Materials and Methods

2.2.1. Study Population and Data Collection

This study utilized a prospective birth cohort from the administrative regions of Sirajdikhan and Pabna Sadar in Bangladesh. The purpose of studying this cohort was to investigate how chronic low dose As exposure affected reproductive outcomes during 2008–2011. A detailed description of the cohort analyzed in this study is available in the literature [43–46]. Briefly, the study recruited participants from areas known to have on average moderate but widely varying As concentrations in the ground water. Health care workers who had been trained by the Dhaka Community Hospital (DCH) Trust and who lived in villages served by DCH rural clinics assisted the researchers in identifying women eligible for the study and inviting them to participate. The eligibility criteria were age ≥18 years, ultrasound confirmed pregnancy at Gestation Week ≤16, use of tubewell-supplied groundwater as the primary drinking water source, intention to live at the current residence for the duration of pregnancy, intention to receive prenatal health care from DCH, and agreement to deliver either at DCH or at home with a DCH-trained midwife. Four visits were scheduled with each participant: time of enrollment

(V1), Gestation Week 28 (V2), time of delivery (V3), and Postpartum Month 1 (V4). Data and sample collection in this study included sociodemographic characteristics (V1); lifestyle and personal habits (V1); medical records (V1, V2, and V4); drinking water history, e.g., drinking water source and water use habits, etc. (V1 and V2); dietary assessment (V2 and V4); toenail samples (V1 and V4); and water samples (V1 and V4). Participation in the study was incentivized by an offer of free prenatal care from DCH, including free multivitamin supplements. Each participant received in-home monthly checkups. At each checkup, multivitamin supplements were replenished and compliance with the prescribed multivitamin regimen was assessed.

Figure 2-1 presents a flowchart of the participants (n = 1616) during the study. The cohort was enrolled between January 2008 and June 2011. Dietary assessment 1 was performed at V2, and dietary assessment 2 was performed at V4. In analysis of dietary assessment 1, the investigators excluded 816 participants due to missing data for toenail As (n = 483), missing data for water As (n = 2), extremely low or extremely high caloric intake defined as <500 kcal/day or >3500 kcal/day, respectively (n = 322), and toenail mass <5 mg (n = 9). In analysis of dietary assessment 2, a total of 725 participants were excluded due to missing data for toenail As (n = 482), missing data for water As (n = 4), extremely low or extremely high caloric intake (n = 226), and toenail mass <5 mg (n = 13). Thus, the final sample size was 800 and 891 for dietary assessment 1 and 2, respectively. Sensitivity analysis was performed using alternative exclusion criteria, i.e., total caloric intake below the 5th percentile and 95th percentile of the entire cohort. Informed consent was obtained from all study participants. The study protocol was approved by the institutional review boards of DCH and Harvard T.H. Chan School of Public Health (HSPH) (IRB number P11351, approved February 2008).

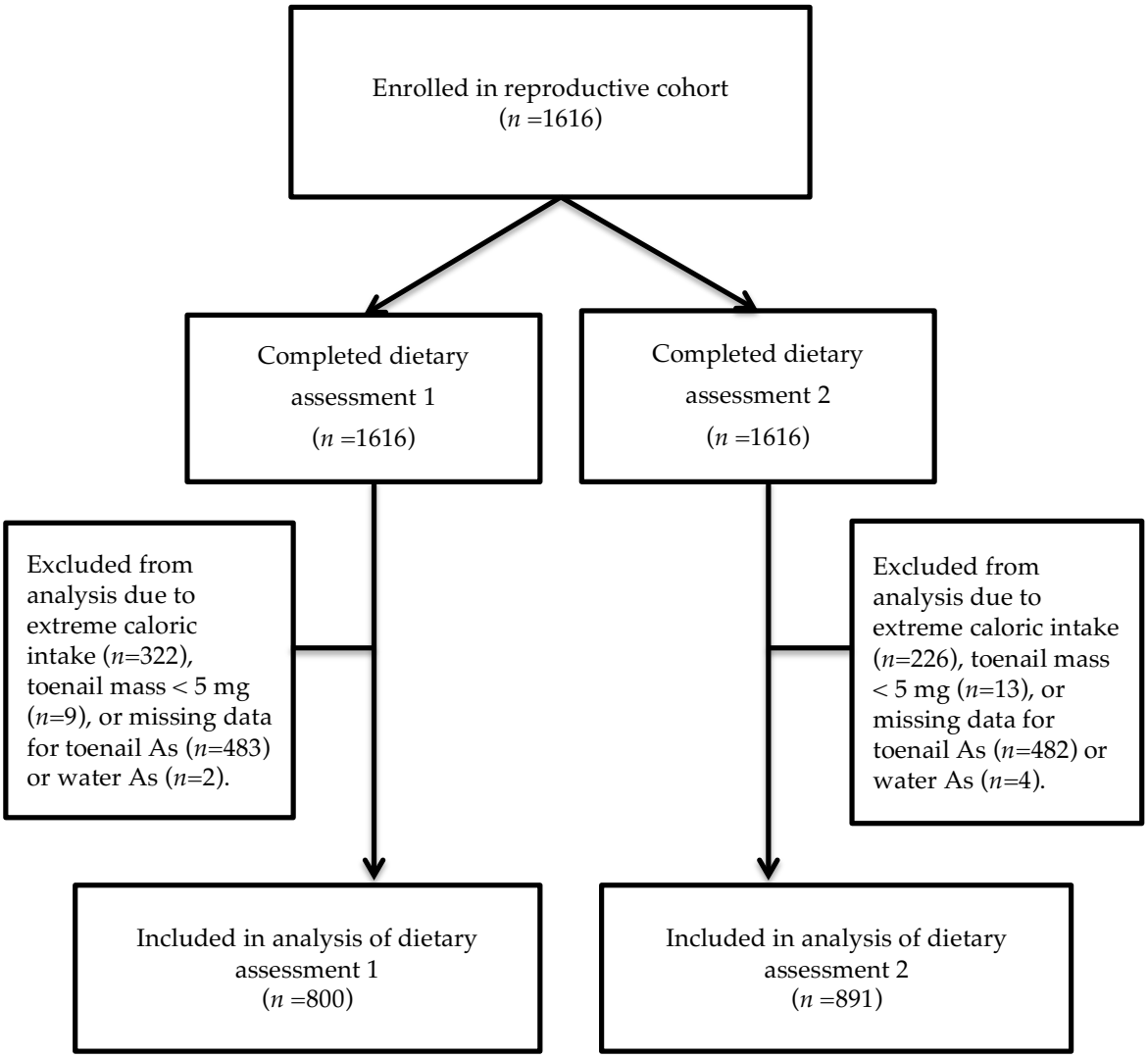

Figure 2-1. Study flow chart.

2.2.2. Dietary Assessment

The FFQ used in this study was a locally-validated semi-quantitative written instrument covering the preceding 12-month period [47]. All participants (n = 1616), with the assistance of trained interviewers, completed the FFQ two times: at Gestation Week 28 (dietary assessment 1) and at Postpartum Month 1 (dietary assessment 2). The FFQ required participants to recall how frequently they had consumed 42 food items common in Bangladesh during the previous year.

The food items were divided into five categories: (1) cereal and bread; (2) vegetables; (3) legumes, pulses, and seeds; (4) fish, poultry, meat, and eggs; and (5) milk based food items. Frequency of consumption was indicated on a 5-point scale ranging from "never" to "daily". Participants were also shown photographs of locally-used plates, bowls, and serving utensils and then asked to indicate their typical portion sizes. Consumption of dietary supplements (e.g., vitamins) was also surveyed. As all participants received multivitamin supplements at V1, multivitamin intake was not analyzed for associations with toenail As concentration. All responses were converted to servings per day and grams per day using the midpoint of each frequency interval and assuming a 30-day month. Foods that were left blank were coded as not consumed [48]. Imputation procedures used in this study were performed as described in the literature [47]. Total energy intake was estimated using the most recent Food Composition Table for Bangladesh [49].

2.2.3. Arsenic Exposure Assessment

Arsenic has a high affinity for sulfhydryl groups and accumulates in keratin-rich tissues such as toenail [50]. Speciation analysis using HPLC-ICP-MS of aqueous toenail extract from other studies showed that As^{III}, As^{V}, and organic dimethylarsinate (DMA^{V}) account for approximately 83%, 13%, and 8.5% of total toenail As concentration, respectively [51]. This study used toenail As as a biomarker of long-term As exposure in the past 2–18 months [32]. Each participant provided a sample of toenail clippings which were analyzed for total As concentrations using previously established protocols [44–46]. Briefly, clippings were collected using stainless steel scissors at V1 and V4, stored in labeled paper envelopes at room temperature, and shipped to HSPH for analysis. The toenail samples were processed by microwave-assisted acid digestion as described in Chen et al. [52]. Total As in the digested

samples was analyzed by inductively coupled plasma (ICP) mass spectrometry (MS; Perkin Elmer, Shelton, CT, USA). A method bank and a certified reference material (CRM, human hair, Shanghai Institute of Nuclear Research, Academia Sinica, Shanghai, China) were included with each batch of samples used for digestion and analysis. Samples from the same study participant were digested and analyzed in the same batch. All analytical values were blank-corrected. To account for inter-batch differences in instrument performance, analytical values were multiplied by a factor equal to the inverse of the batch-specific percentage recovery in the CRM (mean percentage recovery for As was 76%). The mean limit of detection (LOD) for toenail As was 0.04 μg/g, and the relative standard deviation was 6.1%. Statistical comparisons of MS results for the toenail As samples and the CRM samples were performed as described in the literature.

For each participant, samples of the primary source of drinking water were collected in 50 mL polypropylene tubes (BD Falcon, BD Bioscience, Bedford, MA, USA) at V1 and V4 and preserved in reagent grade nitric acid (Merck, Darmstadt, Germany) a pH < 2. The HSPH Environmental Laboratory Services (North Syracuse, New York, NY, USA) used USA EPA method 200.8 to perform ICP-MS of As in all water samples. Quality control testing of the instrument with spiked laboratory control sample (ICP, Analytical Mixture 12 Solution A, High Purity Standard, Charleston, SC, USA) yielded recoveries of 98% to 107%. For water samples that were below the LODs (n = 326 and n = 246 for V1 and V4, respectively), half the value of the LOD was used for statistical analysis.

2.2.4. Statistical Analysis

Data were analyzed using SAS (version 9.3; SAS Institute Inc., Cary, NC, USA). Descriptive statistics were computed for all variables. Our previous study suggested that ingested water was the dominant source of As exposure in areas where the drinking water concentration

exceeded the Bangladesh standard of 50 μg/L [13]. To examine whether water As level affected the association between water-corrected toenail As concentration and the intake level of each consumed food, we used a similar approach as described by Cottingham et al. [32]. Briefly, we included an interaction term between the intake rate of individual food item and an indicator variable for water As concentration (≤50 μg/L vs. > 50 μg/L). For all foods, the interaction was statistically significant ($\alpha = 0.05$), therefore, we grouped participants into high (>50 μg/L) and low exposure (≤50 μg/L) based on the As concentration in their primary source of drinking water. The *t* test, Fisher exact test, or analysis of variance was then used to compare the mean values of all variables between the two groups.

A generalized linear model was used to evaluate the relationship between toenail As concentrations and consumption of individual foods reported in the FFQ. Distributions of water As concentration, toenail As concentration, and individual food intake were skewed. Therefore, these variables were transformed according to their natural log (ln) form before regression modeling. "Crude" associations were considered to be associations between the consumption level of each food item and ln-transformed toenail As concentration after adjusting only for ln-transformed water As concentration within each strata of drinking water As concentration (≤50 μg/L vs. >50 μg/L). "Adjusted" associations accounted for additional covariates, including age, daily water and energy intake (estimated by FFQ), sex, betel nut chewing, tobacco chewing, smoking status, passive exposure to cigarette smoke, body mass index (BMI), and education level. Consumption measured by dietary assessment 1 at V2 was matched with toenail As and water As at V1; consumption measured by dietary assessment 2 at V4 were matched with toenail As and water As collected at V4. Reported effect estimates ($\hat{\beta}$) have units of ln((toenail As concentrations, μg/g)·$(g/d)^{-1}$). For food items that were significantly associated with toenail As

concentration, we tested the robustness of these associations by re-running models after and deleting apparent outliers; all associations remained significant.

To provide a more useful interpretation of parameter estimates, we employed a method previously reported in which we calculated the percentage change in toenail As concentration between the 5th and 95th percentiles of consumption for each consumed food [32]. Specifically, the percentage change in toenail As concentration was estimated using a back-transformation based on those participants who were nonsmokers, had a normal BMI (≥18.5 to <25 kg/m^3), had attained a secondary education, and had a median value for water As concentration, age, water intake, and daily energy intake within their exposure group.

The false discovery rate (FDR) approach was used to account for type I error in conducting multiple statistical tests for each consumed food [53]. The *Q*-value (minimum FDR at which a test result may be considered statistically significant) was calculated from the combined list of *p*-values for the association with each food using the *Q*-value estimation package (Bioconductor version 3.4) in R [54]. A *Q*-value < 0.05 after correcting for multiple testing was considered statistically significant.

For a clear visual depiction of multiple associations, the *corrplot* package in R was used to summarize all slope coefficients of the crude and adjusted models on a feature-expression heat-map [55].

2.3. Results

Among the 800 women in the cohort that completed dietary assessment 1 at V2, the median toenail As concentration was 1.6 μg/g, and the water As concentration ranged from 0.9 μg/L (lower quartile) to 35.3 μg/L (upper quartile) at V1 (Table 2-1). The 891 women who completed dietary assessment 2 at V4 had a median toenail As concentration of 1.2 μg/g, and

water As concentration ranged from 1.3 μg/L (lower quartile) to 48.0 μg/L (upper quartile) at V4. The analysis included all food items in the dish-based FFQ except for three food items that no participant reported consuming (Table S2-1 and Table S2-2). In the sensitivity analysis excluding participants with a daily caloric intake below the 5th percentile or above the 95th percentile of the entire cohort, fewer subjects were excluded from analyses (n = 162 and n = 113 for dietary assessment 1 and 2, respectively), however conclusions were not affected.

2.3.1. Comparison between Low and High Water As Exposure

As expected, mean toenail As concentration was significantly lower in women with low (≤50 μg/L) water As exposure than in women with high (>50 μg/L) water As exposure. For both energy intake and water intake, average daily values were similar at dietary assessment 1 and dietary assessment 2. However, women with high water As had higher daily caloric intake and lower daily water intake compared to women with low water As (Table 2-1). Women with low water As and women with high water As were otherwise similar in age, betel nut use, tobacco chewing, cigarette smoking, BMI, and education level.

2.3.2. Crude versus Adjusted Model

The crude and adjusted generalized linear models yielded similar results for most food items (Figure 2-2, Tables S2-1 and Table S2-2). The directions and strengths of associations were robust to the inclusion of potential confounding variables for individuals with low water As exposure. In individuals with high water As exposure, the directions of associations remained unchanged in comparisons of crude and adjusted models. However, as shown in Figure 2-2, for several food items (i.e., plain rice, meat kebab, and meat with legumes), the strength of the association changed after adjusting for potential confounders.

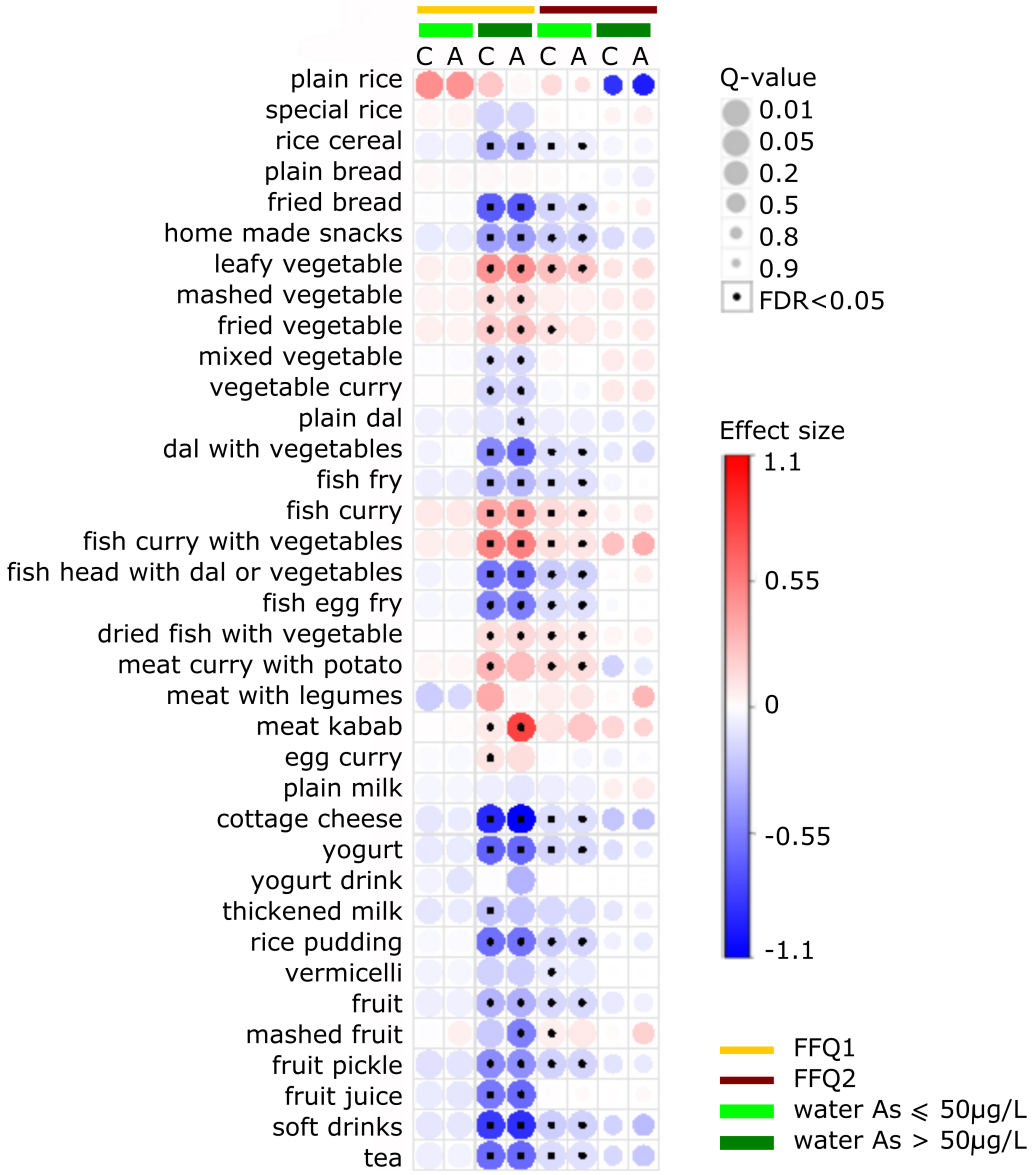

Figure 2-2. Associations between natural log-transformed food intake (g/day) and natural log-transformed toenail As concentration (μg/g) measured by the first and the second dietary assessment (FFQ1 and FFQ2, respectively) among women exposing to drinking water As concentration of >50 μg/L and ≤50 μg/L in Bangladesh. Effect size is the slope coefficients ($\hat{\beta}$) for each dietary item and has the unit of ln((toenail As concentrations, μg/g)·(g/d)$^{-1}$). "C" stands for crude model adjusted for water As concentration only; "A" stands for adjusted model adjusted for water As level, sex, smoking in the living environment, chewing betel nut, BMI, daily water intake, daily energy intake, and education level. *Q*-value accounting for multiple comparisons using the false discovery rate (FDR = 0.05) method. (Quantitative values presented in the Supplementary Materials, Tables S2-1 and Table S2-2).

2.3.3. Comparison of Dietary Assessments 1 and 2

Mean toenail As concentration measured at V4 (at dietary assessment 2) was significantly lower than that at V1 (dietary assessment 1) ($p = 0.05$; Table 2-2). However, water As concentration and average daily caloric intake did not differ significantly between these two time points ($p = 0.443$ and $p = 0.919$, respectively). In dietary assessments 1 and 2, toenail As level showed a similar pattern of associations with average daily intake in all food items except for "plain rice" and "mashed fruit". In subjects with high water As exposure (>50 μg/L), plain rice was positively associated with toenail As in dietary assessment 1 but was negatively associated with toenail As in dietary assessment 2 (Figure 2-2, Tables S2-1 and S2-2). However, no associations were statistically significant after adjustment for multiple testing. In subjects with high water As exposure, mashed fruit also had a significant positive association with toenail As in dietary assessment 1 but had a weak negative association with toenail As in dietary assessment 2. In individuals with low water As exposure (≤50 μg/L), the association between toenail As and consumption of mashed fruit was stronger in dietary assessment 2 than in dietary assessment 1.

2.3.4. Food Items Positively Associated with ln-Transformed Toenail As

Figure 2-2 shows that ln-transformed toenail As concentration had significant positive associations with ln-transformed consumption of plain rice, leafy vegetables, mashed vegetables, fried vegetables, fish curry, fish curry with vegetables, dried fish with vegetables, meat with potatoes, and meat kebab (Tables S2-1 and Table S2-2). Figure 2-2 further shows that, after correction for multiple testing, the positive association remained statistically significant for all food items except plain rice in individuals who had high water As exposure in dietary assessment 1 and in individuals with low water As exposure in dietary assessment 2. In the high water As exposure group, the food item that had the strongest positive association with toenail As was

meat kebab (adjusted model $\hat{\beta} = 0.771 \pm 0.122$, $p < 0.001$ in dietary assessment 1). Table 2-2 shows that, according to this model, an increase in meat kebab consumption from 9 g/day (5th percentile) to 900 g/day (95th percentile) would incur a 33-fold increase in ln-transformed toenail As. In individuals with low water As exposure, the food items that had the strongest associations with toenail As were plain rice (adjusted model $\hat{\beta} = 0.445 \pm 0.213$, $p = 0.037$ in dietary assessment 1) and leafy vegetables (adjusted model $\hat{\beta} = 0.224 \pm 0.057$, $p < 0.001$ in dietary assessment 2), although the positive association for plain rice was not significant after adjusting for multiple comparison (Q-value > 0.05). The percent change in ln-transformed toenail As comparing the 5th to 95th percentile of consumption was predicted to be 13.65% and 36.45% for plain rice and leafy vegetable, respectively (Table 2-2).

2.3.5. Food Items Negatively Associated with ln-Transformed Toenail As

Many food items were negatively associated with toenail As. After correction for multiple comparisons, grains and bread based foods that had significant negative associations with toenail As included rice cereal, fried bread, and homemade snacks. In individuals with high water As exposure, an increase in consumption of rice cereal from 10 g/day (5th percentile) to 75 g/day (95th percentile) predicted a 62% decrease in toenail As. In the categories of legumes and pulses, milk based foods, fruits, and beverages, all food items showed negative associations with toenail As. In individuals with high water As exposure, the food items that had the strongest negative associations with toenail As were cottage cheese (adjusted model $\hat{\beta} = -1.053 \pm 0.150$, $p < 0.001$ in dietary assessment 1), soft drinks (adjusted model $\hat{\beta} = -0.861 \pm 0.149$, $p < 0.001$ in dietary assessment 1), and fried bread (adjusted model $\hat{\beta} = -0.679 \pm 0.167$, $p < 0.001$ in dietary assessment 1).

Table 2-1. Demographic characteristics of the study population across the two dietary assessment periods.

	1st Dietary Assessment (FFQ1)							2nd Dietary Assessment (FFQ2)						
Group	Total (n=800)		Water [As] ≤50µg/L (n=629)		Water [As] >50µg/L (n=171)			Total (n=891)		Water [As] ≤50µg/L (n=683)		Water [As] >50µg/L (n=208)		
A. Continuous Variables	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-value***	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**p-value***
Age (years)	23.0	4.3	23.0	4.3	23.2	4.3	0.590	23.0	4.2	23.0	4.3	23.1	3.9	0.764
Toenail [As] (µg/g)	3.3	4.6	2.3	3.5	7.1	5.8	<0.001	2.7	4.1	1.8	3.3	5.8	4.7	<0.001
Water [As] (µg/L)	45.8	111.6	6.8	11.1	188.9	178.6	<0.001	50.0	113.2	8.6	12.7	210.6	193.1	<0.001
Water intake (L/d)	2.1	0.5	2.2	0.5	2.0	0.4	<0.001	2.2	0.4	2.2	0.4	2.1	0.4	0.002
Energy intake (kcal/day)	2804.2	417.5	2787.6	416.7	2865.0	416.1	0.032	2806.2	396.1	2777.5	389.9	2900.7	402.4	<0.001
B. Dichotomous Variables	**N**	**%**	**N**	**%**	**N**	**%**		**N**	**%**	**N**	**%**	**N**	**%**	
Sex (Female)	800	100	629	100	171	100	1.000	891	100.0	683	100.0	208	100.0	1.000
Betel Nut	6	0.8	4	0.6	2	1.2	0.614	8	0.9	6	0.9	2	1.0	0.591
Chew Tobacco	4	0.5	2	0.3	2	1.2	0.202	6	0.7	4	0.6	2	1.0	0.427
Environmental smoke	339	42.4	251	39.9	88	51.5	0.010	362	40.6	257	37.6	105	50.5	0.001
Smoker	0	0.0	0	0.0	0	0.0	1.000	0	0.0	0	0.0	0	0.0	1.000
C. Categorical Variables	**N**	**%**	**N**	**%**	**N**	**%**		**N**	**%**	**N**	**%**	**N**	**%**	
BMI														
Underweight (<18.5)	231	28.9	173	27.5	58	33.9		241	27.0	168	24.6	73	27.0	
Normal (18.5 ≤ BMI<25)	497	62.1	395	62.8	102	59.6	0.209	569	63.9	447	65.4	122	63.9	0.016
Overweight (25 ≤ BMI<30)	65	8.1	56	8.9	9	5.3		71	8.0	59	8.6	12	8.0	
Obese (≥30)	7	0.9	5	0.8	2	1.2		10	1.1	9	1.3	1	1.1	
Education														
Illiterate	12	1.5	10	1.6	2	1.2		10	1.1	8	1.2	2	1.0	
Able to write	106	13.3	81	12.9	25	14.6		125	14.0	94	13.7	31	14.9	
Primary Education	271	33.9	218	34.7	53	31.0	0.378	292	32.8	236	34.6	56	26.9	0.499
Secondary Education	385	48.1	298	47.4	87	50.9		431	48.4	319	46.7	112	53.9	
Higher Secondary Education	24	3.0	21	3.3	3	1.8		27	3.0	21	3.1	6	2.9	
College/Graduate	1	0.1	0	0.0	1	0.6		4	0.4	3	0.4	1	0.5	
Post-graduate	1	0.1	1	0.2	0	0.0		2	0.2	2	0.3	0	0.0	

* Comparing water [As] ≤50 µg/L vs. >50 µg/L using two sample *t* test for continuous variables, Fisher's exact test for dichotomous variables and Chi-square test for categorical variables.

Table 2-2. Mean daily intake level for each food item and the percent change in toenail As concentration comparing the 5th to the 95th percentile of intake level.

	1st Dietary Assessment						2nd Dietary Assessment					
	Water [As] ≤50ug/L (n=629)			Water [As] >50ug/L (n=171)			Water [As] ≤50ug/L (n=683)			Water [As] >50ug/L (n=208)		
	Daily intake ∞		% change	Daily intake ∞		% change	Daily intake ∞		% change	Daily intake ∞		% change
Food Item	Mean	STD	toenail As⌘	Mean	STD	toenail As⌘	Mean	STD	toenail As⌘	Mean	STD	toenail As⌘
Grain, Cereal, Bread based												
Plain rice *(Bhaat, Panta bhaat)*	1355.14	160.79	13.65	1396.38	151.84	0.79	1331.07	150.00	4.95	1369.2	107.1	-3.22
Special rice *(Khichuri, Pulao, Biriyani)*	25.83	30.67	6.47	25.04	30.21	-26.23	25.51	27.68	-0.48	24.6	23.7	9.89
Rice cereal *(Chira, Muri, Khoi, Murki)*	68.60	56.93	-16.40	29.12	42.02	-62.00*	62.42	52.53	-21.67*	14.5	20.3	-8.43
Plain bread *(Atta ruti, Pau ruti)*	84.60	92.94	6.28	81.20	79.88	7.73	98.22	103.74	-1.84	73.5	65.7	-13.32
Fried bread *(Porota, Luchi)*	22.37	20.17	-3.46	12.67	6.24	-61.80*	33.52	25.52	-27.46*	14.5	12.3	8.76
Home made snacks *(Pitha-puli)*	31.96	32.74	-15.21	17.23	22.38	-61.77*	32.06	25.40	-37.83*	12.1	8.3	-21.34
Vegetable based												
Leafy vegetable *(Sak)*	56.03	41.31	6.29	80.86	41.32	85.95*	54.54	37.07	36.43*	91.5	35.7	9.35
Mashed vegetable *(Bhorta)*	24.87	18.92	13.05	29.70	21.10	59.42*	20.16	20.86	12.59	29.7	21.3	30.41
Fried vegetable *(Bhaji)*	50.82	32.08	15.33	66.77	34.89	97.20*	49.04	31.13	27.93	71.4	36.8	28.30
Mixed vegetable *(Labra)*	41.88	34.47	-4.79	43.66	44.04	-39.72*	35.00	33.09	-0.10	38.7	41.6	31.97
Vegetable Curry (Torkarir jhole)	51.91	36.11	3.13	51.96	51.61	-45.60*	47.47	32.28	-4.94	39.3	44.7	38.48
Legumes, Pulses, Seeds based												
Plain dal	67.82	38.63	-12.83	58.72	42.30	-39.28*	64.22	30.46	-12.07	57.6	37.7	-25.19
Dal with vegetables	10.77	5.00	-0.11	9.99	3.94	-49.19*	44.52	34.67	-28.83*	7.8	9.8	-12.57
Fish, Poultry, Meat, Egg based												
Fish Fry *(Mach bhaji)*	194.79	128.82	-18.61	101.51	101.42	-54.10*	197.75	147.83	-30.76*	56.3	65.7	-1.26
Fish curry *(Mach er jhole)*	126.17	101.19	36.93	211.48	92.26	116.90*	119.67	98.76	47.27*	217.8	83.6	17.76
Fish curry with vegetable	156.39	121.88	21.21	271.76	93.54	189.10*	160.99	128.42	24.30*	301.9	61.3	26.18
Fish head with dal or vegetables	12.64	10.83	-5.62	7.05	5.07	-74.44*	10.98	11.12	-28.62*	5.1	3.3	13.42
Fish egg fry (Maccher dim bhaji)	4.87	4.00	-6.83	2.00	2.44	-77.12*	8.05	8.25	-35.29*	1.3	1.7	0.26
Dried fish with vegetable	94.79	116.21	-1.01	157.31	128.09	64.23*	102.32	129.05	38.37*	210.9	122.9	13.71
Meat curry with potato	32.60	34.24	9.51	36.34	19.05	20.64	24.65	20.37	41.15*	37.3	25.6	-5.22
Meat with legumes *(Halim)*	12.28	4.06	-13.98	12.18	3.69	2.98	16.64	13.17	32.98	23.7	15.2	102.25
Meat kebab	171.36	555.09	9.20	200.26	415.79	3321.24*	96.46	66.16	54.79	130.9	82.9	37.86
Egg curry *(Dim er jhole)*	98.57	84.90	-7.24	134.87	107.91	43.85	96.42	71.96	-8.45	139.9	103.1	-3.31
Milk-based												
Plain milk *(Doodh)*	127.10	92.50	-13.78	81.47	73.91	-32.26	132.31	87.45	-13.33	70.7	58.2	36.04
Cottage cheese *(chana)*	2.45	1.24	-11.58	1.60	0.94	-79.78*	3.88	3.28	-32.93*	0.9	1.0	-28.02
Yogurt *(Doi)*	5.20	4.50	-13.87	2.88	2.66	-71.94*	4.74	3.17	-25.05*	1.7	1.0	-9.96
Yogurt drink *(Ghole, Matha, Borhani)*	7.13	4.89	-21.63	5.97	3.71	-44.92	NA	NA	NA	NA	NA	NA
Thickened milk *(Khoa, kheer)*	11.83	7.83	-10.20	10.08	5.99	-27.50	11.40	4.86	-18.26	9.8	7.5	-15.66
Rice pudding *(Payesh)*	15.07	11.86	-2.96	8.54	5.48	-68.47*	14.85	15.51	-32.05*	6.8	7.2	-9.59
Vermicelli *(Semai)*	30.15	27.62	-9.81	22.91	21.91	-23.35	33.42	30.39	-18.49	20.9	15.4	1.16
Fruits												
Fruit	78.85	62.33	-17.87	32.39	39.69	-58.55*	79.74	55.88	-38.10*	37.3	109.8	-13.78
Mashed fruit *(Bhorta)*	2.32	1.12	7.93	2.53	4.20	-49.13*	5.34	9.08	41.70	9.6	10.1	77.70
Fruit pickle *(Aachar)*	1.67	3.15	-24.05	1.26	1.79	-78.20*	1.95	1.61	-37.58*	0.7	0.6	-15.64
Beverages												
Fruit juice	26.09	19.30	-21.87	12.85	12.28	-76.25*	4071.49	36352.71	15.73	2343.3	18866.8	21.76
Soft drinks	17.33	9.56	-15.11	10.22	5.78	-75.45*	16.42	10.75	-28.95*	7.0	2.3	-20.71
Tea	36.63	27.29	-9.65	21.58	23.80	-77.18*	40.64	31.81	-26.73*	11.7	10.0	-20.03

∞: Unit in ln(gram/day); ⌘: Comparing consumers of 5th percentile of intake with those of 95th percentile of intake for each individual food item. The comparison is done among nonsmoker, with normal BMI and secondary education level at the mean age, water intake, and daily energy intake and the median water As concentration of each exposure group using the beta coefficient estimate of adjusted model (Unit: percent change in g/day); *: *Q*-value < 0.05.

2.4. Discussion

This study used two successive dietary assessments (one at 28 weeks of gestation and the other at one month postpartum) to examine the relationship between long-term dietary patterns and As exposure in pregnant women. Total toenail As concentrations reflect the equilibrium of As distribution between the blood and nail during nail formation and extrusion [56] and provide a recapitulation of As exposure during the 2–18 months before sampling [32,57]. The validated dish-based FFQ used in this study captured dietary patterns in the previous 12 months; Thus, the exposure window captured by the FFQ in this study was matched with the As exposure window measured by the toenail clippings. Associations between food items and toenail As showed similar directions across the two time points. In dietary assessment 1, the associations were strongest among individuals with high water As exposure. In dietary assessment 2, the associations were strongest among individuals with low water As exposure. This difference suggests that accumulation of As through diet might differ before and during pregnancy. The ability of the body to methylate and excrete As is likely to have a positive association with the amount of As available for accumulation in the toenail. Notably, the crude and energy-adjusted regression results were similar, which suggests that, in terms As accumulation in the body, the total quantity of As consumed is more important than the quantity of As consumed in proportion to energy intake or body size. A previous study of the association between diet and toenail As in the US reported similar results [32].

Increased metabolism of As is among the many changes in metabolic physiology that occur during pregnancy to prepare the body for fetal growth. Data for metabolites of urinary As indicate that As methylation efficiency increases during pregnancy. Specifically, the shift from higher concentrations of the toxic As metabolite urinary monomethylarsonous acid

(MMA) to higher concentrations of dimethylarsinous acid (DMA) after As exposure is more pronounced in pregnant women than in the general population [58–62]. Increased As methylation efficiency facilitates excretion of As from the body, which may explain the weaker association found in this study between food items and toenail As concentrations observed at dietary assessment 2 (which indicated dietary habits during pregnancy) compared to dietary assessment 1 (dietary habits prior to pregnancy and at early pregnancy). Another possible explanation for these weaker associations during pregnancy is related to increased folate consumption through the multivitamin supplement given to all participants after enrollment in this study. However, the effect of the supplement was probably small since increased methylation efficiency during pregnancy occurs independently of folate status [60]. Consumption of folate-related nutrients also affects As metabolism in the body. Studies in Bangladesh have reported that high intake of folate, cysteine, methionine, and calcium is associated with reduced urinary iAs [63] and with reduced incidence of As-related skin lesions [64]. During pregnancy, the low water As exposure group showed stronger associations between consumption of various food items and toenail As compared to the high water As exposure group. This suggests that changes in methylation efficiency and mechanisms of As removal from the body may differ between high and low As exposure. Further studies are needed to clarify how different levels of As exposure affect methylation efficiency during pregnancy.

The population analyzed in this study represented a wide range of water As exposure levels. A previous study in Bangladesh suggested that food is a large contributor to total As exposure in areas with high As levels in the ground water (>50 μg/L) [13]. The current study similarly observed this interaction between water As exposure and diet. Thus, the study

population was stratified into two groups based on water As exposure level. Compared to individuals with low water As exposure, those with high water As exposure tended to consume more rice, vegetables, fish, eggs, and meat but less rice cereal, homemade bread, and milk based food items. The difference in consumption levels did not explain the difference in the strength of associations between the two groups since the generalized linear model accounted for differences in consumption levels. However, the difference in strength of associations does suggest that As exposure affects As metabolism in a nonlinear fashion.

Arsenic concentration in food is dependent on aspects of the environment in which crops are cultivated, including location and season. For example, rice cultivated in As-contaminated soil and water showed higher level of As concentration, and rice crops irrigated with ground water showed significantly higher concentrations than rice cultivated at monsoon season [4,5]. The current study evaluated the long-term diet habit over the past 12 months, thus the effect of seasonal changes cannot be evaluated. Stratification of the analytical results by geographic area revealed no significant associations, possibly due to the lack of statistical power.

Elevated As levels in rice have been documented in several countries, including Bangladesh [4,5,15,65,66], the United States [67–69], and other countries in Asia [33,70]. Inorganic As contents in rice increased significantly after cooking. For example, a simulated gastrointestinal digestion study showed that iAs in cooked rice has 63%–99% bioavailability [71]. A longitudinal study of 18,470 adults in Bangladesh revealed a positive association between steamed rice consumption and creatinine-adjusted urinary total As, and similar findings were reported for a USA population and a Bangladeshi community living in the

United Kingdom [21,25,72,73]. An FFQ survey performed in the USA further revealed a significant correlation between rice consumption and toenail As.

When the analyses in the current study focused only on the data for dietary assessment 1 (i.e., when data during pregnancy were excluded), rice consumption had a positive, albeit not statistically significant, association with toenail As. The association was stronger in participants exposed to low water As levels. In this group, an increase in rice consumption from the 5th to the 95th percentile predicted a 13.65% increase in toenail As concentration in nonsmokers who had a normal BMI, secondary education level, mean age, mean daily water and energy intake, and median water As concentration. Notably, toenail As was negatively associated with consumption of other grains, cereals and cereal products such as rice cereal, fried bread and homemade snacks. Possible explanations for this negative association include the low water content and low As level in these food items as well as the lower consumption of rice relative to consumption of grains, cereal, and bread. There is a need for further study to determine whether these food items are effective and feasible alternatives to plain rice in areas known to have As-contaminated water.

Vegetables, fish and meat items also revealed positive associations with toenail As. Laboratory analysis of vegetables commonly included in the Bangladeshi diet, such as *kachu sak* (*Colocasia antiquorum*), potatoes (*Solanum tuberisum*), and *kalmi sak* (*Ipomoea reptoms*), showed elevated As levels in these food items. However, the elevation of As varied by cultivar and area of cultivation [31]. Another survey performed in Shanxi, China, found that 77% of ground water samples exceeded the WHO guideline of 10 μg/L As. Several vegetables revealed As levels exceeding 1 μg/g, including cucumbers, tomatoes, eggplant, scallions, beans, and cabbage [33]. In a New Hampshire population known to have As-contaminated

drinking water, Brussels sprouts consumption was positively associated with toenail As level [32]. The total As concentration in vegetables, predominately iAs, varies by the plant tissue type [74]. Generally, the order of As accumulation in plant tissues is, from first to last, root, stem, leaf, and grain. In vivo feeding studies show that the As bioavailability of edible plant tissues ranges from 45% (chard) to 95% (mung bean) [74]. Available data for the association between vegetable consumption and As exposure are limited. A Pakistan study reported that iAs exposure from vegetable consumption is not a major cancer risk factor [75]. While our data showed that some vegetable food items (leafy vegetables, mashed vegetables, and fried vegetables) are positively associated with toenail As, the association is likely attributable to the high water As content of the vegetable items or their preparation method. Further studies are needed for a more comprehensive understanding of the association between vegetable consumption and As uptake.

The positive association between fish consumption and toenail As is poorly understood. In the USA, fish and seafood consistently show higher total As concentrations compared to other food items and are the main contributors to As intake [26,29,76,77]. Recent studies show that consumption of fish is positively associated with As biomarkers in both urine [77,78] and toenail tissue [56]. A possible explanation for this association is that, although iAs contributes a relatively small percentage of total As in seafood (approximately 1.5% and 20% in fish and shellfish, respectively), the iAs in seafood may still contribute appreciably to total As exposure [25]. New evidence also suggests that reactive trivalent arsenic intermediates, which have high affinity for the cysteine component in nails, may be formed during the bioconversion of arsenosugars and arsenolipids in seafood to urinary excretion of DMA [79]. However, more research is needed to clarify the possible mechanism behind this.

Consumptions of fruits and most milk based food items were negatively associated with toenail As. Fruits are important sources of micronutrients, fiber, and antioxidants (e.g., vitamins E and C), which protect the body from oxidative stress induced by As exposure [80]. Therefore, consumption of more fruits may result in lower toenail As due to better overall nutrition. Another explanation for these negative associations may be related to factors which mitigate As absorption in the gastrointestinal tract [25], and which could be the case for milk based food items that contain high levels of fat and protein. A study in a New Hampshire population with low groundwater As was the first to report that dietary lipids are negatively associated with toenail As [81]. In another study, an in vitro simulation of the human gastrointestinal microbial system showed that a typically high fat, high protein western diet results in lower As bioaccessibility compared with a typical high fiber, low fat Asian diet (63.4% versus 81.2%, respectively) [82]. Animal models show that interactions between As and invertebrate and vertebrate lipid particles cause As detoxification via cell-free or sequestration mechanisms [83]. Additional studies are needed to clarify the roles of lipid consumption in As detoxification and As accumulation in the human body.

One strength of this study is the use of a validated FFQ [47]. In comparison with the six-day food diary conducted a previous study [47], the FFQ showed fair to strong correlations for grain (Spearman correlation = 0.35); vegetables (Spearman correlation = 0.25); legumes, pulses, and seeds (Spearman correlation = 0.34); fish, poultry, meat, and eggs (Spearman correlation = 0.42); milk (Spearman correlation = 0.66); and fruits (Spearman correlation = 0.79). Another strength is the use of a prospective cohort design, which revealed the temporal sequence of exposure and outcome and avoided selection bias at enrollment. This study evaluated heavy metals exposure not only by performing a comprehensive dietary assessment,

but also by collecting water samples during each household visit; by administering comprehensive questionnaires on lifestyle, family medical history, and water use; and by collecting biospecimens. Third, a rigorously-developed gold standard ICP-MS method was used to detect toenail As concentration. Comprehensive comparisons with other As biomarkers show that toenail As has good sensitivity and specificity [12,45]. Finally, analysis of a population representing a wide range of As exposure levels provided adequate power to observe associations with As exposure.

In large epidemiologic studies, the FFQ is considered the most feasible way to capture long-term dietary habits [84]. However, using a FFQ to estimate consumption is subject to important sources of error. The average daily energy intake in the current study was higher than that reported by the Bangladesh Household Income and Expenditure Survey (HIES) [85]. Preferred food items or food items with known health benefits are likely to be over-reported in an FFQ survey [47,86]. In the current study, the higher average daily energy intake obtained by the FFQ in comparison with the HIES probably resulted from high consumption of plain rice, which is the main source of caloric intake in rural Bangladesh. Additionally, the subjects of this study may have tended to over-report consumption of rice because it symbolizes better economic status. Nevertheless, considering that most participants had similar socioeconomic status (most had a secondary education or lower), over-reporting was expected to be non-differential and was therefore expected to bias the association toward the null. This over-reporting bias may also explain the small variation in plain rice consumption observed in our study population, which may in turn contribute to the non-significant association observed between rice consumption and toenail As concentration.

Another limitation of the FFQ is its relative lack of precision in comparison with other dietary assessment methods. Potentially more precise instruments, such as the diet record or 24-h recall, are available but were not feasible in this study due to their laboriousness, cost, and unsuitability for measuring long-term intake. While we matched the exposure window captured by the FFQ with the As exposure window measured by the toenail clippings, use of the FFQ may still incur recall bias, such that one's recent diet may influence reporting of consumption levels. In comparison with precision of the toenail As level, the precision of dietary measurements was lower, violating the convention that predictor variables should be more precise than the response variable in regression modeling [32]. Fortunately, this violation tends to bias results toward the null, so our findings should still be valid [87].

2.5. Conclusions

In this study, toenail As concentration, which is a well-recognized biomarker of As exposure, showed positive associations with several food items commonly consumed in Bangladesh, including leafy vegetables (*sak*), mashed vegetables (*bhorta*), fried vegetables (*bhaji*), fish curry (*mac her jhole*), fish curry with vegetables, dried fish with vegetables, meat with potatoes, and meat kebab. In contrast, consumption of rice cereal (*chira*, *muri*, *khoi*, and *murki*), fruits, and many milk based food items were negatively associated with toenail As, possibly due to disrupted As absorption in the gastrointestinal tract or due to reduced As intake. Another possible explanation is that these negative associations indicate a specific dietary pattern (i.e., one marked by low consumption of plain rice). The directions of association were similar in groups with exposure to high As in drinking water and groups with exposure to low As in drinking water, however, the strengths of associations markedly differed between these groups. Comparisons of the strength of association at different stages of

pregnancy suggested a change in the mechanisms of As detoxification and excretion from the body during pregnancy. Further studies are needed to explore mechanisms behind the effects of diet on As absorption and excretion and on As metabolism during pregnancy.

2.6. References

1. WHO Exposure to Arsenic: A Major Public Health Concern. Available obline: http://www.who.int/ipcs/assessment/public_health/arsenic/en/ (accessed on 15 April 2015).
2. Agency for Toxic Substances and Disease Registry (ATSDR). *Agency for Toxic Substances and Disease Registry: Arsenic Toxicity: Environmental Alert*; ATSDR: Atlanta, GA, USA, 2000.
3. Tseng, C.-H. A review on environmental factors regulating arsenic methylation in humans. *Toxicol. Appl. Pharmacol.* **2009**, *235*, 338–350.
4. Meharg, A.A.; Rahman, M.M. Arsenic contamination of Bangladesh paddy field soils: Implications for rice contribution to arsenic consumption. *Environ. Sci. Technol.* **2003**, *37*, 229–234.
5. Duxbury, J.M.; Mayer, A.B.; Lauren, J.G.; Hassan, N. Food chain aspects of arsenic contamination in Bangladesh: Effects on quality and productivity of rice. *J. Environ. Sci. Health A Toxic Hazard. Subst. Environ. Eng.* **2003**, *38*, 61–69.
6. MacIntosh, D.L.; Spengler, J.D.; Ozkaynak, H.; Tsai, L.; Ryan, P.B. Dietary exposures to selected metals and pesticides. *Environ. Health Perspect.* **1996**, *104*, 202–209.
7. Pizarro, I.; Gómez, M.M.; Fodor, P.; Palacios, M.A.; Cámara, C. Distribution and biotransformation of arsenic species in chicken cardiac and muscle tissues. *Biol. Trace Elem. Res.* **2004**, *99*, 129–143.
8. Lasky, T.; Sun, W.; Kadry, A.; Hoffman, M.K. Mean total arsenic concentrations in chicken 1989–2000 and estimated exposures for consumers of chicken. *Environ. Health Perspect.* **2004**, *112*, 18–21.

9. Cubadda, F.; Jackson, B.P.; Cottingham, K.L.; Van Horne, Y.O.; Kurzius-Spencer, M. Human exposure to dietary inorganic arsenic and other arsenic species: State of knowledge, gaps and uncertainties. *Sci. Total Environ.* **2017**, *579*, 1228–1239.
10. Borak, J.; Hosgood, H.D. Seafood arsenic: Implications for human risk assessment. *Regul. Toxicol. Pharmacol.* **2007**, *47*, 204–212.
11. European Food Safety Authority. Scientific Opinion on Arsenic in Food. *EFSA J.* **2009**, *7*, 1351.
12. Kile, M.L.; Houseman, E.A.; Breton, C.V.; Quamruzzaman, Q.; Rahman, M.; Mahiuddin, G.; Christiani, D.C. Association between total ingested arsenic and toenail arsenic concentrations. *J. Environ. Sci. Health A Toxic Hazard. Subst. Environ. Eng.* **2007**, *42*, 1827–1834.
13. Kile, M.L.; Houseman, E.A.; Breton, C.V.; Smith, T.; Quamruzzaman, Q.; Rahman, M.; Mahiuddin, G.; Christiani, D.C. Dietary arsenic exposure in bangladesh. *Environ. Health Perspect.* **2007**, *115*, 889–893.
14. Bae, M.; Watanabe, C.; Inaoka, T.; Sekiyama, M.; Sudo, N.; Bokul, M.H.; Ohtsuka, R. Arsenic in cooked rice in Bangladesh. *Lancet* **2002**, *360*, 1839–1840.
15. Alam, M.G.M.; Snow, E.T.; Tanaka, A. Arsenic and heavy metal contamination of vegetables grown in Samta village, Bangladesh. *Sci. Total Environ.* **2003**, *308*, 83–96.
16. Roychowdhury, T.; Tokunaga, H.; Ando, M. Survey of arsenic and other heavy metals in food composites and drinking water and estimation of dietary intake by the villagers from an arsenic-affected area of West Bengal, India. *Sci. Total Environ.* **2003**, *308*, 15–35.
17. Signes, A.; Mitra, K.; Burló, F.; Carbonell-Barrachina, A.A. Effect of cooking method and rice type on arsenic concentration in cooked rice and the estimation of arsenic dietary

intake in a rural village in West Bengal, India. *Food Addit. Contam. A* **2008**, *25*, 1345–1352.

18. Gray, P.J.; Conklin, S.D.; Todorov, T.I.; Kasko, S.M. Cooking rice in excess water reduces both arsenic and enriched vitamins in the cooked grain. *Food Addit. Contam. A* **2016**, 33, 78–85.
19. Carey, M.; Jiujin, X.; Gomes Farias, J.; Meharg, A.A.; Meharg, A.; Williams, P.; Adamako, E.; Lawgali, Y.; Deacon, C.; Villada, A.; et al. Rethinking Rice Preparation for Highly Efficient Removal of Inorganic Arsenic Using Percolating Cooking Water. *PLoS ONE* **2015**, *10*, e0131608.
20. Cascio, C.; Raab, A.; Jenkins, R.O.; Feldmann, J.; Meharg, A.A.; Haris, P.I. The impact of a rice based diet on urinary arsenic. *J. Environ. Monit.* **2011**, *13*, 257–265.
21. Gilbert-Diamond, D.; Cottingham, K.L.; Gruber, J.F.; Punshon, T.; Sayarath, V.; Gandolfi, A.J.; Baker, E.R.; Jackson, B.P.; Folt, C.L.; Karagas, M.R. Rice consumption contributes to arsenic exposure in US women. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 20656–20660.
22. Wei, Y.; Zhu, J.; Nguyen, A. Rice consumption and urinary concentrations of arsenic in US adults. *Int. J. Environ. Health Res.* **2014**, *24*, 459–470.
23. Tabata, H.; Anwar, M.; Horai, S.; Ando, T.; Nakano, A.; Wakamiya, J.; Koriyama, C.; Nakagawa, M.; Yamada, K.; Akiba, S. Toenail arsenic levels among residents in Amami-Oshima Island, Japan. *Environ. Sci.* **2006**, *13*, 149–160.
24. Dabeka, R.W.; McKenzie, A.D.; Lacroix, G.M.; Cleroux, C.; Bowe, S.; Graham, R.A.; Conacher, H.B.; Verdier, P. Survey of arsenic in total diet food composites and estimation of the dietary intake of arsenic by Canadian adults and children. *J. AOAC Int.* **1993**, *76*,

14–25.

25. MacIntosh, D.L.; Williams, P.L.; Hunter, D.J.; Sampson, L.A.; Morris, S.C.; Willett, W.C.; Rimm, E.B. Evaluation of a food frequency questionnaire-food composition approach for estimating dietary intake of inorganic arsenic and methylmercury. *Cancer Epidemiol. Biomarkers Prev.* **1997**, *6*, 1043–1050.
26. Schoof, R.A.; Yost, L.J.; Eickhoff, J.; Crecelius, E.A.; Cragin, D.W.; Meacher, D.M.; Menzel, D.B. A market basket survey of inorganic arsenic in food. *Food Chem. Toxicol.* **1999**, *37*, 839–846.
27. Meharg, A.A.; Deacon, C.; Campbell, R.C.J.; Carey, A.-M.; Williams, P.N.; Feldmann, J.; Raab, A. Inorganic arsenic levels in rice milk exceed EU and US drinking water standards. *J. Environ. Monit.* **2008**, *10*, 428–431.
28. Meharg, A.A.; Sun, G.; Williams, P.N.; Adomako, E.; Deacon, C.; Zhu, Y.-G.; Feldmann, J.; Raab, A. Inorganic arsenic levels in baby rice are of concern. *Environ. Pollut.* **2008**, *152*, 746–749.
29. Tao, S.S.-H.; Michael Bolger, P. Dietary arsenic intakes in the United States: FDA Total Diet Study, September 1991–December 1996. *Food Addit. Contam.* **1999**, *16*, 465–472.
30. DeCastro, B.R.; Caldwell, K.L.; Jones, R.L.; Blount, B.C.; Pan, Y.; Ward, C.; Mortensen, M.E.; Chen, C.; Hsueh, Y.; Lai, M.; et al.. Dietary Sources of Methylated Arsenic Species in Urine of the United States Population, NHANES 2003–2010. *PLoS ONE* **2014**, *9*, e108098.
31. Das, H.K.; Mitra, A.K.; Sengupta, P.K.; Hossain, A.; Islam, F.; Rabbani, G.H. Arsenic concentrations in rice, vegetables, and fish in Bangladesh: A preliminary study. *Environ. Int.* **2004**, *30*, 383–387.

32. Cottingham, K.L.; Karimi, R.; Gruber, J.F.; Zens, M.S.; Sayarath, V.; Folt, C.L.; Punshon, T.; Morris, J.S.; Karagas, M.R. Diet and toenail arsenic concentrations in a New Hampshire population with arsenic-containing water. *Nutr. J.* **2013**, *12*, 149.
33. Cui, J.; Shi, J.; Jiang, G.; Jing, C. Arsenic Levels and Speciation from Ingestion Exposures to Biomarkers in Shanxi, China: Implications for Human Health. *Environ. Sci. Technol.* **2013**, *47*, 5419–5424.
34. Hood, R.D.; Vedel-Macrander, G.C.; Zaworotko, M.J.; Tatum, F.M.; Meeks, R.G. Distribution, metabolism, and fetal uptake of pentavalent arsenic in pregnant mice following oral or intraperitoneal administration. *Teratology* **1987**, *35*, 19–25.
35. Hood, R.D.; Vedel, G.C.; Zaworotko, M.J.; Tatum, F.M.; Meeks, R.G. Uptake, distribution, and metabolism of trivalent arsenic in the pregnant mouse. *J. Toxicol. Environ. Health* **1988**, *25*, 423–434.
36. Flora, S.J.S. Arsenic-induced oxidative stress and its reversibility. *Free Radic. Biol. Med.* **2011**, *51*, 257–281.
37. Wlodarczyk, B.J.; Bennett, G.D.; Calvin, J.A.; Finnell, R.H. Arsenic-induced neural tube defects in mice: Alterations in cell cycle gene expression. *Reprod. Toxicol.* 1**996**, *10*, 447–454.
38. Quansah, R.; Armah, F.A.; Essumang, D.K.; Luginaah, I.; Clarke, E.; Marfo, K.; Cobbina, S.J.; Nketiah-Amponsah, E.; Namujju, P.B.; Obiri, S.; et al. Association of Arsenic with Adverse Pregnancy Outcomes-Infant Mortality: A Systematic Review and Meta-Analysis. *Environ. Health Perspect.* **2015**, *123*, 412–421.
39. Guan, H.; Piao, F.; Zhang, X.; Li, X.; Li, Q.; Xu, L.; Kitamura, F.; Yokoyama, K. Prenatal exposure to arsenic and its effects on fetal development in the general population of Dalian.

Biol. Trace Elem. Res. **2012**, *149*, 10–15.

40. Xu, L.; Yokoyama, K.; Tian, Y.; Piao, F.-Y.; Kitamura, F.; Kida, H.; Wang, P. Decrease in birth weight and gestational age by arsenic among the newborn in Shanghai, China. *Nihon Koshu Eisei Zasshi* **2011**, *58*, 89–95.
41. Smith, A.H.; Hopenhayn-Rich, C.; Bates, M.N.; Goeden, H.M.; Hertz-Picciotto, I.; Duggan, H.M.; Wood, R.; Kosnett, M.J.; Smith, M.T. Cancer risks from arsenic in drinking water. *Environ. Health Perspect.* **1992**, *97*, 259–267.
42. Kordas, K.; Lönnerdal, B.; Stoltzfus, R.J. Interactions between nutrition and environmental exposures: Effects on health outcomes in women and children. *J. Nutr.* **2007**, *137*, 2794–2797.
43. Huyck, K.L.; Kile, M.L.; Mahiuddin, G.; Quamruzzaman, Q.; Rahman, M.; Breton, C.V.; Dobson, C.B.; Frelich, J.; Hoffman, E.; Yousuf, J.; et al. Maternal Arsenic Exposure Associated With Low Birth Weight in Bangladesh. *J. Occup. Environ. Med.* **2007**, *49*, 1097–1104.
44. Kile, M.L.; Rodrigues, E.G.; Mazumdar, M.; Dobson, C.B.; Diao, N.; Golam, M.; Quamruzzaman, Q.; Rahman, M.; Christiani, D.C. A prospective cohort study of the association between drinking water arsenic exposure and self-reported maternal health symptoms during pregnancy in Bangladesh. *Environ. Health* **2014**, *13*, 29.
45. Rodrigues, E.G.; Kile, M.; Dobson, C.; Amarasiriwardena, C.; Quamruzzaman, Q.; Rahman, M.; Golam, M.; Christiani, D.C. Maternal–infant biomarkers of prenatal exposure to arsenic and manganese. *J. Expo. Sci. Environ. Epidemiol.* **2015**, *25*, 639–648.
46. Kile, M.L.; Cardenas, A.; Rodrigues, E.; Mazumdar, M.; Dobson, C.; Golam, M.; Quamruzzaman, Q.; Rahman, M.; Christiani, D.C. Estimating Effects of Arsenic Exposure

During Pregnancy on Perinatal Outcomes in a Bangladeshi Cohort. *Epidemiology* **2016**, *27*, 173–181.

47. Lin, P.-I.; Bromage, S.; Mostofa, M.; Allen, J.; Oken, E.; Kile, M.; Christiani, D. Validation of a Dish-Based Semiquantitative Food Questionnaire in Rural Bangladesh. *Nutrients* **2017**, *9*, 49.
48. Michels, K.B.; Willett, W.C. Self-administered semiquantitative food frequency questionnaires: Patterns, predictors, and interpretation of omitted items. *Epidemiology* **2009**, *20*, 295–301.
49. Shaheen, N.; Rahim, A.T.; Banu, M.C.P.; Bari, L.; Tukun, B.; Mannan, M.; Bhattacharjee, L.; Stadlmayr, B. *Food Composition Table for Bangladesh*; University of Dhaka: Dhaka, Bangladesh, 2013.
50. Karagas, M.R.; Morris, J.S.; Weiss, J.E.; Spate, V.; Baskett, C.; Greenberg, E.R. Toenail samples as an indicator of drinking water arsenic exposure. *Cancer Epidemiol. Biomarkers Prev.* **1996**, *5*, 849–852.
51. Button, M.; Jenkin, G.R. T.; Harrington, C.F.; Watts, M.J. Human toenails as a biomarker of exposure to elevated environmental arsenic. *J. Environ. Monit.* **2009**, *11*, 610–617.
52. Chen, K.L.; Amarasiriwardena, C.J.; Christiani, D.C. Determination of total arsenic concentrations in nails by inductively coupled plasma mass spectrometry. *Biol. Trace Elem. Res.* **1999**, *67*, 109–125.
53. Catelan, D.; Biggeri, A. Multiple testing in disease mapping and descriptive epidemiology. *Geospat. Health* **2010**, *4*, 219–229.
54. Bass, Jds.; Dabney, A.; Robinson, D. Qvalue: Q-Value Estimation for False Discovery Rate Control. R Packag. version 2.6.0, 2015. Available online:

http://github.com/jdstorey/qvalue/ (accessed on October 1 2015).

55. Haarman, B.C.M.; Riemersma-Van der Lek, R.F.; Nolen, W.A.; Mendes, R.; Drexhage, H.A.; Burger, H. Feature-expression heat maps—A new visual method to explore complex associations between two variable sets. *J. Biomed. Inform.* **2015**, *53*, 156–161.
56. Rees, J.R.; Sturup, S.; Chen, C.; Folt, C.; Karagas, M.R. Toenail mercury and dietary fish consumption. *J. Expo. Sci. Environ. Epidemiol.* **2007**, *17*, 25–30.
57. Chen, K.-L.B.; Amarasiriwardena, C.J.; Christiani, D.C. Determination of total arsenic concentrations in nails by inductively coupled plasma mass spectrometry. *Biol. Trace Elem. Res.* **1999**, *67*, 109–125.
58. Hopenhayn, C.; Huang, B.; Christian, J.; Peralta, C.; Ferreccio, C.; Atallah, R.; Kalman, D. Profile of urinary arsenic metabolites during pregnancy. *Environ. Health Perspect.* **2003**, *111*, 1888–1891.
59. Lindberg, A.-L.; Ekström, E.-C.; Nermell, B.; Rahman, M.; Lönnerdal, B.; Persson, L.-Å.; Vahter, M. Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh. *Environ. Res.* **2008**, *106*, 110–120.
60. Gardner, R.M.; Nermell, B.; Kippler, M.; Grandér, M.; Li, L.; Ekström, E.-C.; Rahman, A.; Lönnerdal, B.; Hoque, A.M.W.; Vahter, M. Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. *Reprod. Toxicol.* **2011**, *31*, 210–218.
61. Gardner, R.M.; Engström, K.; Bottai, M.; Hoque, W.A. M.; Raqib, R.; Broberg, K.; Vahter, M. Pregnancy and the methyltransferase genotype independently influence the arsenic methylation phenotype. *Pharmacogenet. Genom.* **2012**, *22*, 508–516.
62. Laine, J.E.; Bailey, K.A.; Rubio-Andrade, M.; Olshan, A.F.; Smeester, L.; Drobná, Z.;

Herring, A.H.; Stýblo, M.; García-Vargas, G.G.; Fry, R.C. Maternal arsenic exposure, arsenic methylation efficiency, and birth outcomes in the Biomarkers of Exposure to ARsenic (BEAR) pregnancy cohort in Mexico. *Environ. Health Perspect.* **2015**, *123*, 186–192.

63. Heck, J.E.; Gamble, M.V.; Chen, Y.; Graziano, J.H.; Slavkovich, V.; Parvez, F.; Baron, J.A.; Howe, G.R.; Ahsan, H. Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. *Am. J. Clin. Nutr.* **2007**, *85*, 1367–1374.
64. Melkonian, S.; Argos, M.; Chen, Y.; Parvez, F.; Pierce, B.; Ahmed, A.; Islam, T.; Ahsan, H. Intakes of several nutrients are associated with incidence of arsenic-related keratotic skin lesions in Bangladesh. *J. Nutr.* **2012**, *142*, 2128–2134.
65. Van Geen, A.; Zheng, Y.; Cheng, Z.; He, Y.; Dhar, R.K.; Garnier, J.M.; Rose, J.; Seddique, A.; Hoque, M.A.; Ahmed, K.M. Impact of irrigating rice paddies with groundwater containing arsenic in Bangladesh. *Sci. Total Environ.* **2006**, *367*, 769–777.
66. Williams, P.N.; Islam, M.R.; Adomako, E.E.; Raab, A.; Hossain, S.A.; Zhu, Y.G.; Feldmann, J.; Meharg, A.A. Increase in Rice Grain Arsenic for Regions of Bangladesh Irrigating Paddies with Elevated Arsenic in Groundwaters. *Environ. Sci. Technol.* **2006**, *40*, 4903–4908.
67. Nriagu, J.O.; Lin, T.-S. Trace metals in wild rice sold in the United States. *Sci. Total Environ.* **1995**, *172*, 223–228.
68. Williams, P.N.; Price, A.H.; Raab, A.; Hossain, S.A.; Feldmann, J.; Meharg, A.A. Variation in Arsenic Speciation and Concentration in Paddy Rice Related to Dietary Exposure. *Environ. Sci. Technol.* **2005**, *39*, 5531–5540.
69. Williams, P.N.; Raab, A.; Feldmann, J.; Meharg, A.A. Market Basket Survey Shows

Elevated Levels of As in South Central USA Processed Rice Compared to California: Consequences for Human Dietary Exposure. *Environ. Sci. Technol.* **2007**, *41*, 2178–2183.

70. Basu, A.; Mitra, S.; Chung, J.; Guha Mazumder, D.N.; Ghosh, N.; Kalman, D.; von Ehrenstein, O.S.; Steinmaus, C.; Liaw, J.; Smith, A.H. Creatinine, Diet, Micronutrients, and Arsenic Methylation in West Bengal, India. *Environ. Health Perspect.* **2011**, *119*, 1308–1313.
71. Laparra, J.M.; Vélez, D.; Barberá, R.; Farré, R.; Montoro, R. Bioavailability of Inorganic Arsenic in Cooked Rice: Practical Aspects for Human Health Risk Assessments. *J. Agric. Food Chem.* **2005**, *53*, 8829–8833.
72. He, Y.; Zheng, Y. Assessment of in vivo bioaccessibility of arsenic in dietary rice by a mass balance approach. *Sci. Total Environ.* **2010**, *408*, 1430–1436.
73. Davis, M.A.; Mackenzie, T.A.; Cottingham, K.L.; Gilbert-Diamond, D.; Punshon, T.; Karagas, M.R. Rice Consumption and Urinary Arsenic Concentrations in USA Children. *Environ. Health Perspect.* **2012**, *120*, 1418–1424.
74. Jean, J.-S.; Bundschuh, Jochen; Bhattacharya, P. Arsenic in Geosphere and Human Diseases, (Arsenic 2010), In Proceedings of the 3rd International Congress on Arsenic in the Environment (As-2010), Taina, Taiwan 17–21 May 2010; CRC Press: London, UK; New York, NY, USA, 2010.
75. Rehman, Z.U.; Khan, S.; Qin, K.; Brusseau, M.L.; Shah, M.T.; Din, I. Quantification of inorganic arsenic exposure and cancer risk via consumption of vegetables in southern selected districts of Pakistan. *Sci. Total Environ.* **2016**, *550*, 321–329.
76. Xue, J.; Zartarian, V.; Wang, S.-W.; Liu, S.V.; Georgopoulos, P. Probabilistic Modeling of Dietary Arsenic Exposure and Dose and Evaluation with 2003–2004 NHANES Data.

Environ. Health Perspect. **2009**, *118*, 345–350.

77. Awata, H.; Linder, S.; Mitchell, L.E.; Delclos, G.L. Association of Dietary Intake and Biomarker Levels of Arsenic, Cadmium, Lead, and Mercury among Asian Populations in the USA: NHANES 2011–2012. *Environ. Health Perspect.* **2017**, *125*, 314–323.
78. Gagnon, F.; Lampron-Goulet, E.; Normandin, L.; Langlois, M.-F. Measurements of Arsenic in the Urine and Nails of Individuals Exposed to Low Concentrations of Arsenic in Drinking Water From Private Wells in a Rural Region of Québec, Canada. *J. Environ. Health* **2016**, *78*, 76–83.
79. Molin, M.; Ulven, S.M.; Meltzer, H.M.; Alexander, J. Arsenic in the human food chain, biotransformation and toxicology—Review focusing on seafood arsenic. *J. Trace Elem. Med. Biol.* **2015**, *31*, 249–259.
80. Slotnick, M.J.; Meliker, J.R.; Kannan, S.; Nriagu, J.O. Effects of nutritional measures on toenail arsenic concentration as a biomarker of arsenic exposure. *Biomarkers* **2008**, *13*, 451–466.
81. Gruber, J.F.; Karagas, M.R.; Gilbert-Diamond, D.; Bagley, P.J.; Zens, M.S.; Sayarath, V.; Punshon, T.; Morris, J.S.; Cottingham, K.L. Associations between toenail arsenic concentration and dietary factors in a New Hampshire population. *Nutr. J.* **2012**, *11*, 45.
82. Alava, P.; Du Laing, G.; Tack, F.; De Ryck, T.; Van De Wiele, T. Westernized diets lower arsenic gastrointestinal bioaccessibility but increase microbial arsenic speciation changes in the colon. *Chemosphere* **2015**, *119*, 757–762.
83. Rahman, M.M.; Rahman, F.; Sansom, L.; Naidu, R.; Schmidt, O. Arsenic interactions with lipid particles containing iron. *Environ. Geochem. Health* **2009**, *31*, 201–206.
84. Willet, W. Food Frequency Methods. In *Nutritional Epidemiology*; Oxford University

Press: Oxford, UK, 2013; pp. 71–72.

85. World Bank. *Bangladesh—Household Income and Expenditure Survey: Key Findings and Results 2010*; World Bank: Washington, DC, USA, 2011.
86. Willett, W. *Nutritional Epidemiology: CH 13 Issues in Analysis and Presentation of Dietary Data*; Oxford Scholarship: Oxford, UK, 2012.
87. Draper, N.R.; Smith, H. *Applied Regression Analysis*; John Wiley & Sons, Inc.: Hoboken, NJ, USA, 1998.

2.7. Supplemental Material

Table S2-1. The association of food intake with toenail arsenic concentration using linear regression for the 1st dietary assessment

	Water [As] ≤50ug/L (n=629)			Water [As] >50ug/L (n=171)		
	Crude[1]	**Adjusted**[2]	**% change**	**Crude**[1]	**Adjusted**[2]	**% change**
Food intake	$\hat{\beta}$(SE)[3]	$\hat{\beta}$(SE)[3]		$\widehat{\boldsymbol{\beta}}(\boldsymbol{SE})$[3]	$\widehat{\boldsymbol{\beta}}(\boldsymbol{SE})$[3]	
Grain, Cereal, Bread based						
Plain rice *(Bhaat, Panta bhaat)*	0.453(0.205)	0.445(0.213)	13.65	0.230(0.681)	0.027(0.714)	0.79
Special rice *(Khichuri, Pulao, Biriyani)*	0.042(0.056)	0.045(0.057)	6.47	-0.169(0.109)	-0.150(0.111)	-26.23
Rice cereal *(Chira, Muri, Khoi, Murki)*	-0.054(0.025)	-0.050(0.025)	-16.40	-0.297(0.049)**	-0.284(0.051)**	-62.00
Plain bread *(Atta ruti, Pau ruti)*	0.030(0.032)	0.025(0.035)	6.28	0.028(0.066)	0.027(0.071)	7.73
Fried bread *(Porota, Luchi)*	-0.007(0.050)	-0.018(0.055)	-3.46	-0.673(0.154)**	-0.679(0.167)**	-61.80
Home made snacks *(Pitha-puli)*	-0.075(0.039)	-0.064(0.041)	-15.21	-0.398(0.076)**	-0.4(000.076)**	-61.77
Vegetable based						
Leafy vegetable *(Sak)*	0.055(0.051)	0.044(0.053)	6.29	0.439(0.106)**	0.447(0.109)**	85.95
Mashed vegetable *(Bhorta)*	0.045(0.031)	0.045(0.032)	13.05	0.147(0.066)*	0.172(0.067)**	59.42
Fried vegetable *(Bhaji)*	0.057(0.039)	0.052(0.040)	15.33	0.210(0.084)**	0.250(0.089)**	97.20
Mixed vegetable *(Labra)*	-0.009(0.035)	-0.014(0.036)	-4.79	-0.141(0.065)*	-0.148(0.068)**	-39.72
Vegetable Curry (Torkarir jhole)	0.004(0.042)	0.011(0.043)	3.13	-0.183(0.067)**	-0.178(0.069)**	-45.60
Legumes, Pulses, Seeds based						
Plain dal	-0.059(0.039)	-0.050(0.039)	-12.83	-0.105(0.072)	-0.146(0.076)*	-39.28
Dal with vegetables	-0.051(0.082)	-0.001(0.088)	-0.11	-0.474(0.195)**	-0.608(0.203)**	-49.19
Fish, Poultry, Meat, Egg based						
Fish Fry *(Mach bhaji)*	-0.068(0.032)	-0.066(0.032)	-18.61	-0.285(0.063)**	-0.286(0.065)**	-54.10
Fish curry *(Mach er jhole)*	0.087(0.034)	0.092(0.036)	36.93	0.368(0.104)**	0.382(0.115)**	116.90
Fish curry with vegetable	0.070(0.034)	0.071(0.036)	21.21	0.504(0.108)**	0.524(0.111)**	189.10
Fish head with dal or vegetables	-0.043(0.046)	-0.029(0.047)	-5.62	-0.561(0.101)**	-0.574(0.101)**	-74.44
Fish egg fry (Maccher dim bhaji)	-0.039(0.033)	-0.022(0.035)	-6.83	-0.507(0.075)**	-0.530(0.077)**	-77.12
Dried fish with vegetable	0.006(0.024)	-0.002(0.026)	-1.01	0.135(0.058)*	0.149(0.062)*	64.23
Meat curry with potato	0.038(0.039)	0.033(0.041)	9.51	0.300(0.147)*	0.271(0.159)	20.64
Meat with legumes *(Halim)*	-0.201(0.107)	-0.154(0.147)	-13.98	0.338(0.241)	0.030(0.335)	2.98
Meat with grains, legumes, vegetables (*Dhansak*)	-	-	-	-	-	-
Meat kabab	0.002(0.021)	0.017(0.057)	9.20	0.085(0.048)*	0.771(0.122)**	3321.24
Egg curry *(Dim er jhole)*	-0.016(0.033)	-0.022(0.034)	-7.24	0.113(0.069)*	0.134(0.080)	43.85
Milk based						
Plain milk *(Doodh)*	-0.040(0.029)	-0.039(0.030)	-13.78	-0.073(0.064)	-0.102(0.069)	-32.26
Cottage cheese *(chana)*	-0.104(0.065)	-0.074(0.075)	-11.58	-0.874(0.144)**	-1.053(0.150)**	-79.78
Yogurt *(Doi)*	-0.089(0.047)	-0.077(0.049)	-13.87	-0.636(0.090)**	-0.618(0.092)**	-71.94
Yogurt drink *(Ghole, Matha, Borhani)*	-0.043(0.048)	-0.114(0.077)	-21.63	-0.003(0.116)	-0.307(0.176)	-44.92
Thickened milk *(Khoa, kheer)*	-0.096(0.079)	-0.078(0.082)	-10.20	-0.245(0.134)*	-0.236(0.138)	-27.50
Rice pudding *(Payesh)*	-0.024(0.048)	-0.014(0.049)	-2.96	-0.581(0.108)**	-0.577(0.112)**	-68.47
Vermicelli *(Semai)*	-0.049(0.045)	-0.039(0.046)	-9.81	-0.182(0.116)	-0.192(0.120)	-23.35
Sweetmeats (*Mishti*)	-	-	-	-	-	-
Fruits						
Fruit	-0.070(0.029)	-0.063(0.031)	-17.87	-0.300(0.062)**	-0.316(0.063)**	-58.55
Mashed fruit *(Bhorta)*	-0.002(0.074)	0.057(0.089)	7.93	-0.217(0.143)	-0.519(0.163)**	-49.13
Fruit pickle *(Aachar)*	-0.116(0.046)	-0.111(0.046)	-24.05	-0.465(0.085)**	-0.457(0.086)**	-78.20
Beverages						
Fruit juice	-0.081(0.042)	-0.099(0.043)	-21.87	-0.548(0.089)**	-0.612(0.095)**	-76.25
Soft drinks	-0.104(0.061)	-0.097(0.067)	-15.11	-0.809(0.145)**	-0.861(0.149)**	-75.45
Tea	-0.067(0.044)	-0.043(0.046)	-9.65	-0.602(0.094)**	-0.619(0.095)**	-77.18
Coffee	-	-	-	-	-	-

[1] "Crude" indicates the model with adjusted for water arsenic concentration only.

[2] "Adjusted" indicates the model with adjustment for water arsenic level, sex, smoking in the living environment, chewing betel nut, BMI, daily water intake, daily energy intake, and education level.

[3] Units for the estimated coefficients are natural-log transformed (toenail arsenic concentration, μg/g)*(g/day)$^{-1}$. Q-value accounting for multiple comparisons using the false discovery rate (FDR=0.05) method (** Q-value <0.01; * Q-value <0.05)

Table S2-2 The association of food intake with toenail arsenic concentration using linear regression for the 2nd dietary assessment

	Water [As] ≤50ug/L (n=683)			Water [As] >50ug/L (n=208)		
	Crude	**Adjusted[a]**	**% change**	**Crude**	**Adjusted[a]**	**% change**
Food intake	$\widehat{\boldsymbol{\beta}}(\boldsymbol{SE})$	$\widehat{\boldsymbol{\beta}}(\boldsymbol{SE})$		$\widehat{\boldsymbol{\beta}}(\boldsymbol{SE})$	$\widehat{\boldsymbol{\beta}}(\boldsymbol{SE})$	
Grain, Cereal, Bread based						
Plain rice *(Bhaat, Panta bhaat)*	0.146(0.258)	0.119(0.258)	4.95	-0.850(0.701)	-0.935(0.722)	-3.22
Special rice *(Khichuri, Pulao, Biriyani)*	0.012(0.066)	-0.003(0.066)	-0.48	0.046(0.081)	0.068(0.089)	9.89
Rice cereal *(Chira, Muri, Khoi, Murki)*	-0.077(0.025)**	-0.071(0.026)*	-21.67	-0.042(0.053)	-0.032(0.054)	-8.43
Plain bread *(Atta ruti, Pau ruti)*	0.015(0.038)	-0.007(0.039)	-1.84	-0.040(0.054)	-0.071(0.062)	-13.32
Fried bread *(Porota, Luchi)*	-0.179(0.047)**	-0.148(0.050)**	-27.46	0.040(0.122)	0.064(0.136)	8.76
Home made snacks *(Pitha-puli)*	-0.207(0.042)**	-0.186(0.043)**	-37.83	-0.141(0.079)	-0.120(0.083)	-21.34
Vegetable based						
Leafy vegetable *(Sak)*	0.254(0.054)**	0.224(0.057)**	36.43	0.103(0.102)	0.129(0.110)	9.35
Mashed vegetable *(Bhorta)*	0.059(0.031)	0.044(0.032)	12.59	0.083(0.050)	0.098(0.053)	30.41
Fried vegetable *(Bhaji)*	0.125(0.046)**	0.091(0.048)	27.93	0.061(0.066)	0.092(0.074)	28.30
Mixed vegetable *(Labra)*	0.023(0.035)	0.000(0.035)	-0.10	0.075(0.047)	0.083(0.049)	31.97
Vegetable Curry (Torkarir jhole)	-0.031(0.043)	-0.024(0.043)	-4.94	0.085(0.052)	0.095(0.054)	38.48
Legumes, Pulses, Seeds based						
Plain dal	-0.067(0.049)	-0.063(0.050)	-12.07	-0.087(0.055)	-0.085(0.058)	-25.19
Dal with vegetables	-0.132(0.030)**	-0.113(0.031)**	-28.83	-0.087(0.099)	-0.137(0.104)	-12.57
Fish, Poultry, Meat, Egg based						
Fish Fry *(Mach bhaji)*	-0.131(0.030)**	-0.118(0.032)**	-30.76	-0.042(0.063)	-0.005(0.068)	-1.26
Fish curry *(Mach er jhole)*	0.139(0.035)**	0.113(0.037)**	47.27	0.044(0.083)	0.081(0.086)	17.76
Fish curry with vegetable	0.135(0.036)**	0.105(0.037)*	24.30	0.247(0.119)	0.336(0.133)	26.18
Fish head with dal or vegetables	-0.215(0.052)**	-0.187(0.053)**	-28.62	0.019(0.101)	0.063(0.106)	13.42
Fish egg fry (Maccher dim bhaji)	-0.138(0.028)**	-0.122(0.03)**	-35.29	-0.024(0.087)	0.001(0.090)	0.26
Dried fish with vegetable	0.098(0.023)**	0.079(0.025)**	38.37	0.042(0.051)	0.047(0.064)	13.71
Meat curry with potato	0.168(0.047)**	0.127(0.048)*	41.15	-0.183(0.126)	-0.077(0.139)	-5.22
Meat with legumes *(Halim)*	0.064(0.041)	0.101(0.088)	32.98	0.014(0.072)	0.285(0.177)	102.25
Meat with grains, legumes, vegetables (*Dhansak*)	-	-	-	-	-	-
Meat kabab	0.107(0.058)	0.222(0.112)	54.79	0.160(0.093)	0.179(0.232)	37.86
Egg curry *(Dim er jhole)*	-0.001(0.043)	-0.032(0.044)	-8.45	-0.048(0.053)	-0.012(0.064)	-3.31
Milk based						
Plain milk *(Doodh)*	-0.065(0.037)	-0.058(0.038)	-13.33	0.057(0.049)	0.081(0.052)	36.04
Cottage cheese *(chana)*	-0.134(0.033)**	-0.132(0.038)**	-32.93	-0.233(0.104)	-0.255(0.155)	-28.02
Yogurt *(Doi)*	-0.179(0.049)**	-0.152(0.05)**	-25.05	-0.136(0.135)	-0.080(0.142)	-9.96
Yogurt drink *(Ghole, Matha, Borhani)*	-	-	-	-	-	-
Thickened milk *(Khoa, kheer)*	-0.157(0.081)	-0.142(0.086)	-18.26	-0.099(0.092)	-0.063(0.095)	-15.66
Rice pudding *(Payesh)*	-0.203(0.047)**	-0.175(0.048)**	-32.05	-0.053(0.096)	-0.077(0.100)	-9.59
Vermicelli *(Semai)*	-0.093(0.041)*	-0.075(0.042)	-18.49	0.000(0.092)	0.008(0.096)	1.16
Sweetmeats (*Mishti*)	-	-	-	-	-	-
Fruits						
Fruit	-0.162(0.031)**	-0.154(0.032)**	-38.10	-0.088(0.050)	-0.054(0.054)	-13.78
Mashed fruit *(Bhorta)*	0.059(0.028)*	0.086(0.044)	41.70	0.013(0.053)	0.189(0.091)	77.70
Fruit pickle *(Aachar)*	-0.184(0.040)**	-0.170(0.042)**	-37.58	-0.108(0.105)	-0.085(0.110)	-15.64
Beverages						
Fruit juice	0.003(0.015)	0.019(0.045)	15.73	0.003(0.025)	0.031(0.085)	21.76
Soft drinks	-0.205(0.055)**	-0.188(0.058)**	-28.95	-0.183(0.182)	-0.274(0.204)	-20.71
Tea	-0.141(0.038)**	-0.124(0.039)**	-26.73	-0.164(0.138)	-0.226(0.146)	-20.03
Coffee	-	-	-	-	-	-

[1] "Crude" indicates the model with adjusted for water arsenic concentration only.
[2] "Adjusted" indicates the model with adjustment for water arsenic level, sex, smoking in the living environment, chewing betel nut, BMI, daily water intake, daily energy intake, and education level.
[3] Units for the estimated coefficients are natural-log transformed (toenail arsenic concentration, μg/g)*(g/day)$^{-1}$.
Q-value accounting for multiple comparisons using the false discovery rate (FDR=0.05) method (** Q-value <0.01; * Q-value <0.05).

Chapter 3. Evaluating the potential mediating role of arsenic in the relationship between maternal diet and birth outcomes in rural Bangladesh

Abstract: Epidemiological evidence suggested arsenic exposure during pregnancy might reduce birth weight of the infant. As diet alone can be a significant source of arsenic exposure, arsenic may indirectly affect infant growth by mediating the effect of maternal diet on birth weight (BW). This study utilized a large prospective birth cohort of 1057 eligible pregnant women in Bangladesh to explore the relationship between diet, arsenic exposure and birth outcomes, including BW, gestational age at birth (GAB), and gestational weight gain (GWG), using a causal mediation analysis. Total energy and macronutrient intakes were calculated using data self-reported by a validated semi-quantitative food-frequency questionnaire. Arsenic exposure level was estimated by maternal toenail arsenic concentration. Effect estimates for exposure-mediator, exposure-outcome, and mediator-outcome relationships were calculated using multiple linear regressions and mediation analyses, which employed a counterfactual approach, were performed. Analyses were performed both with and without energy-adjustment. Multiple linear regression showed total energy intake was not significantly associated with toenail arsenic nor BW, in contrast, when decomposing diet intake into macronutrients and using other birth outcomes, higher absolute and energy-adjusted protein, fat and fiber intakes were associated with higher toenail arsenic and lower GAB and GWG, while higher absolute and energy-adjusted carbohydrate intake was associated with lower toenail arsenic and greater GAB and GWG. Mediation analysis showed significant natural indirect effects by toenail arsenic in the relationship between absolute fat, carbohydrate and fiber intake on GAB, specifically 3% (95% CI: 1%-6%) of the association between carbohydrate intake and GAB was mediated by change in toenail arsenic level, while the mediating effect of toenail

arsenic was 6% (95% CI: 1%-9%) for absolute fat intake and 10% (95% CI: 4%-13%) for absolute fiber intake. After adjusting for total energy, no significant mediating effect was found, suggesting the absolute amount of arsenic exposure rather than the arsenic level in relationship to total energy intake was a more important factor to consider when understanding the negative implication of arsenic on fetal growths. In conclusion, though we did not observe a mediating role of arsenic in the association between maternal diet and BW in this study population, higher toenail arsenic associated with higher absolute fat and fiber intake significantly mediated the reduction of GAB, while lower toenail arsenic associated with higher absolute carbohydrate intake significantly mediate the increase of GAB. The study supports the hypothesis that absolute intake of arsenic mediates the effect of maternal diet on GAB.

Keywords: arsenic exposure; gestational age at birth; gestational weight gain; birth weight; fetal growth; preterm birth; mediation analysis; maternal diet; food frequency questionnaire; rural Bangladesh

3.1. Introduction

Arsenic, a ubiquitous naturally occurring metalloid, is one of the top 10 chemicals of public health concern announced by the World Health Organization (WHO) impacting more than 300 million people in the world (1,2). Many countries have reported elevated water arsenic concentrations, including Argentina, China, Mongolia, Taiwan, Bangladesh, Nepal, Chile, India, Mexico, Poland, Vietnam, and the USA(3),; however, the situation in Bangladesh is most devastating (4). Ground water surveys estimated that 57 million people in Bangladesh

are exposed to ground water arsenic exceeding the WHO guideline of 10μg/L and that 35 million are exposed to level above the Bangladeshi government recommendation of 50μg/L (5). In addition to contaminated water, arsenic exposure can occur through consumption of contaminated food. Compared to arsenic exposure from water, dietary source of arsenic exposure, in contrast, had received relatively less attention. In areas with lower arsenic level in the ground water, dietary intake has been identified the major source of arsenic exposure (6,7). High levels of arsenic have been reported in several foods including fish, meat, poultry, bakery good and cereals, and fat and oils (8). Agricultural products can accumulate arsenic from contaminated soil, water, and pesticides (9–11), while arsenic in livestock may come from feed, feed supplement, and foraged grass and plants (9–14). One study estimated that the daily exposure to inorganic arsenic was 1.68-3.00 μg/kg bw/day for adults in Bangladesh, which is much higher than most countries in Europe and North America (15).

Arsenic is a known carcinogen (16–21) and may increase the risk of other noncancerous diseases including skin lesion, blackfoot disease, diabetes, hypertension and chronic obstructive pulmonary disease (22–24). Recent evidences suggested arsenic as a suspected reproductive toxicant as it may readily cross the placental barrier from mother to fetus. Systematic reviews of population-based studies indicated high levels of arsenic in ground water (≥ 50 μg/L) to be associated with greater risk of spontaneous abortion, stillbirth, moderate risk of neonatal mortality and infant mortality (25), and most recent studies investigating low to moderate level of arsenic exposure indicated that arsenic is associated with lower birth weight (BW) (25–29), gestational age(29,30), birth length, and chest and head circumferences (28). Bangladesh has a high prevalence of preterm birth and low birth weight (LBW). An active surveillance from 2004 to 2007 showed that 21% of the newborns were

preterm (<37 weeks of gestation) and 55.3% had low birth weight (LBW, <2500 gram), making it the country with the highest incidence of LBW babies in the world(31). Birth weight is an important indicator of child survival(32) and may be associated with developmental problems in childhood and risk of various disease in adulthood(33). Factors influencing BW in developing countries include pregnancy complications, maternal nutritional status before and during pregnancy, maternal diet, and exposure to specific toxic agents, including smoke, infection, and possibly arsenic (34). Our study group established a birth cohort during 2008 to 2011 to prospectively investigate the effect of chronic arsenic exposure on the health of pregnant women and their offspring in an area of Bangladesh where a moderate range of arsenic level was found in the drinking water source. Data from this cohort suggested lower birth weight associated with elevated maternal total arsenic exposure in a dose-dependent manner(30,35).

Maternal diet may affect birth outcomes via different mechanisms. While the composition of macronutrients influences maternal physiology and metabolism and contributes to the required building blocks for fetal development (36–38), food intake may also be the vehicle delivering and increasing exposure to environmental toxicants,. Furthermore, food composition and toxicants may interact with each other to influence each others' effects on determining health outcomes, such as by altering the amount of toxicants bioavailable or by altering nutrient metabolism. The interaction of environmental exposure to toxicants and maternal nutrient intake is complex and not well investigated(37). One of the best known example of this interaction is demonstrated with fish consumption, which contains both beneficial nutrients such as docosahexaenoic acid (DHA), an n-3 fatty acids that are required for brain and retinal development, and methylmercury, a toxicant that contributes to adverse neurodevelopmental

effects (39–41). The interaction of arsenic exposure and nutrients had mainly focused on the role of folate on arsenic metabolism, and most studies were conducted on undernourished population (42–45). The interaction of arsenic exposure and maternal diet, and their effects on birth outcomes has not been clearly explored. Preliminary analysis (unpublished) from our cohort of pregnant women showed that higher intakes of protein, fat and fiber were associated with lower birth weights. Since evidences from this cohort suggested that maternal consumption of certain food dishes, including vegetable, fish, and meat were associated with higher toenail arsenic level among pregnant women (46). We suspected that part of the adverse effect on BW might be mediated through elevated cumulative arsenic exposure level. To separate the direct effect of maternal diet of total energy consumption and macronutrients on BW and the indirect effect mediated via increasing arsenic exposure, we employed a causal mediation model (47) to investigate level of mediation by total arsenic exposure, as measured by maternal toenail arsenic, in the relationship between maternal diet and BW. Our previous analysis using structural equation modeling suggested that the effect of arsenic on birth weight (BW) was mediated via decreasing gestational age at birth (GAB) and reducing gestational weight gain (GWG) (30), thus, we also explored these two birth outcomes as while testing the interaction of maternal diet and toenail arsenic.

3.2. Materials and Methods

3.2.1. Study Population and Data Collection

Our study enrolled 1,613 pregnant women between 2008 to 2011 for a prospective birth cohort study in Sirajdikhan and Pabna Sadar Upazilas of Bangladesh where the arsenic level in ground water was moderate yet ranged widely. Detail description of the cohort had been

described previously (30,35,48,49). Briefly, pregnant women age ≥18 years of age were approached by local health care workers trained by Dhaka Community Hospital (DCH) Trust and written informed consents were obtained when the women agreed to participate in the study. To be eligible for the study, subjects must have ultrasound confirmed pregnancy of ≤16 weeks' gestation, use a tube well that supplied groundwater as the primary drinking water source, plan to live at the current residence for the duration of the pregnancy, plan to continue prenatal health care with DCH, and agree to deliver at DCH or at home with a DCH-trained midwife. All women received free prenatal care from DCH and were provided with free multivitamin supplement upon enrollment. In additional to three scheduled visits throughout pregnancy (at enrollment, 28 weeks of gestational age, and one month postpartum), trained health care workers paid monthly follow-up visits to women's homes? to distribute prenatal vitamins; to record vitamin compliance, blood pressure, and health symptoms; and to measure weight by scale and height by stadiometor (30). Pregnant women self-reported their sociodemographic characteristics, and habits using structured questionnaires administered at the time of enrollment. The questionnaire included a multiple-choice question on physical activities level: "sitting most of the day" was considered as low, "on feet all day but in stationary position or only spend half day moving" as medium, and "on feet all day and constantly moving" as high physical activity level. The study protocol was approved by the institutional review boards of DCH and Harvard T.H. Chan School of Public Health (HSPH) (IRB number P11351, approved February 2008).

This analysis used all eligible women with singleton livebirth (n=1184). After excluding pregnant women with missing variables (n=92), toenail mass <5 mg (n=14), and extreme daily energy intake <5th and >95th percentile (n=21), the sample size was 1,057 (Figure 3-1).

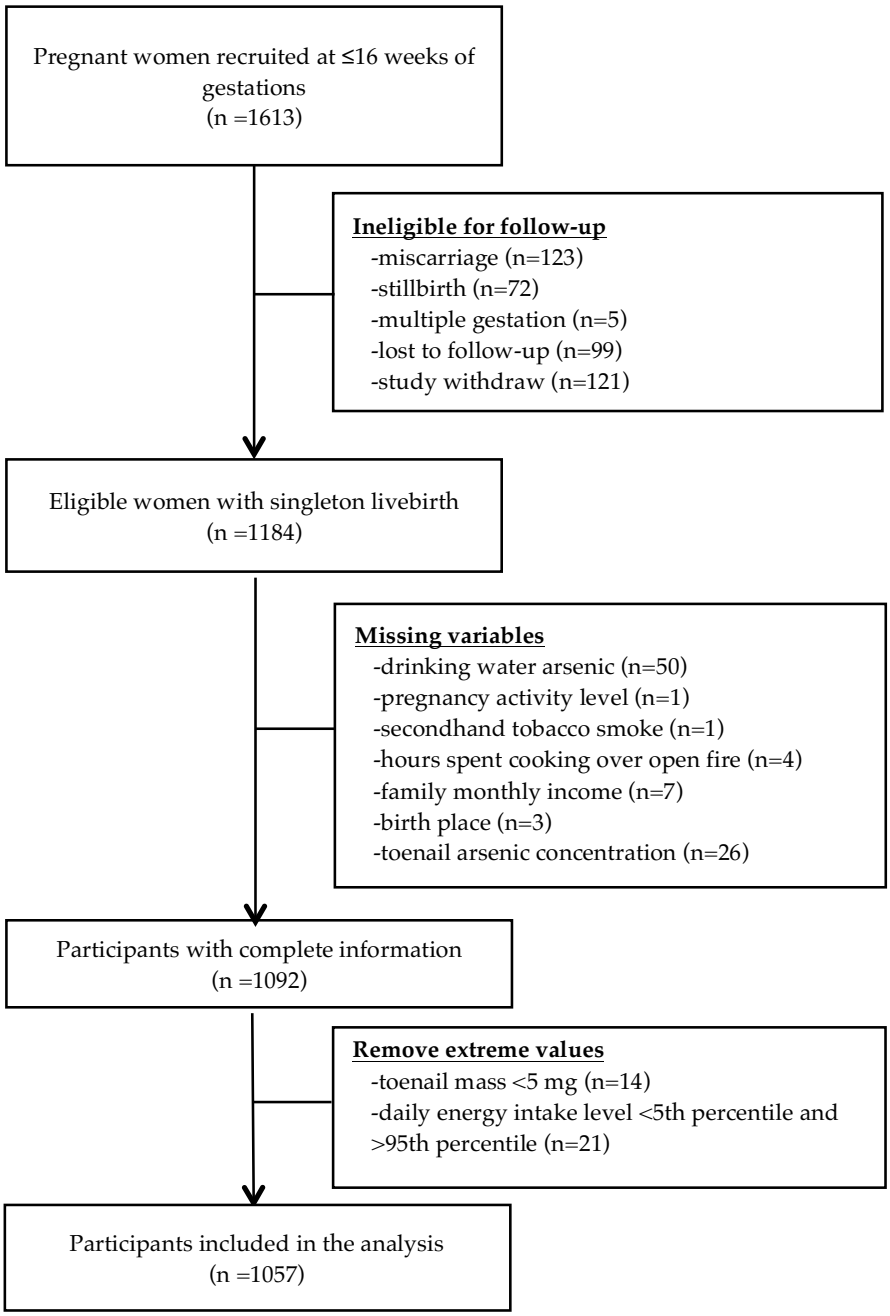

Figure 3-1. Study flow chart

3.2.2 Analysis of Arsenic Exposure

Toenail arsenic has been widely used as a long-term biomarker of arsenic exposure in the previous 2-18 months (50,51). The study used toenail clippings collected at one-month postpartum to retrospectively estimate the arsenic exposure level accumulated throughout pregnancy. The method of collecting toenail clippings has been described previously (49). Toenail samples were acid-digested by microwave using the method described in Chen et al (52), then total arsenic concentration in the digested samples was analyzed by inductively coupled plasma (ICP) mass spectrometry (MS; Perkin Elmer, Shelton, CT, USA) (30,48,49). We included a method blank and a certified reference material (CRM, human hair, Shanghai Institute of Nuclear Research, Academia Sinica, China) in each batch of digestion and analysis sample. All reported analytical values were blank-corrected. Inter-batch differences in instrument performance were accounted for by multiplying the analytical values by the inverse of the batch-specific percentage recovery in the CRM (mean percentage recovery for arsenic was 76%). The mean limit of detection (LOD) for toenail arsenic was 0.04 μg/g, and the relative standard deviation (s.d.) was 6.1%.

Arsenic exposure from water was assessed using the water samples from the primary drinking source provided by each participant at one month postpartum. We used 50mL polypropylene tubes (BD Falcon, BD Bioscience, Bedford, MA) to collect water samples, and added reagent grade nitric acid (Merck, Germany) to adjusted pH <2 for preservation purpose. Water samples were shipped to Environmental Laboratory Services (North Syracuse, NY, USA) to be analyzed by ICP-MS using the US EPA method 200.8. The instruments had recoveries of 98% to 107% when tested with spiked laboratory control (ICP, Analytical Mixture 12 Solution A, High Purity Standard, Charleston, SC, USA). We assigned half of the

LOD to water samples that were below the LODs (n= 246).

3.2.3 Assessment of energy and macronutrient intakes

We used a locally validated dish-based semi- quantitative food frequency questionnaire (FFQ) (53) to collect dietary information. Using the FFQ administered at one month postpartum health care workers guided Pregnant women to recall their dietary habits during the previous 12-months. The method to calculate total energy and nutrient intakes were described previously (46,53). Briefly, consumption of each food item (gram per day) was calculated by multiplying frequency, quantity and portion size. Item left blank were treated as no consumption and missing frequency, quantity or portion size were imputed with the median value using method described in the literature (53,54). Total energy and nutrient intakes were estimated using the nutritional values in the most recent Food Composition Table for Bangladesh (55). The FFQ has been validated against two 3-day food diaries with reasonable correlations observed for total energy (Spearman r=0.35), protein (Spearman r=0.46), fat (Spearman r=0.45), carbohydrate (Spearman r=0.50), and fiber (Spearman r=0.43) between food diaries and the FFQ (53).

3.2.4 Assessment of fetal growth factors and other covariates

All women received ultrasound examination at the time of enrollment and at the 2nd trimester to estimate gestational age. GAB was recorded by trained health care workers at the time of delivery. Maternal prenatal body weights were measured at monthly follow-ups by health care workers using a calibrated scale and GWG was calculated as a function of lb/week using the estimated slope of the linear regression model between monthly weight and gestational week from the 14th week to the last available weight measurement prior to

delivery. Birth weight was measured by trained health care worker using a pediatric scale that was calibrated before each use to measure birth weight at the location of delivery (45% measured at a hospital or clinic and 55% at participant's home) and rounded to the nearest 10g. Newborn sex, birth delivery location, and birth delivery type were recorded at the time of delivery by the research staff using standardized reporting form. Other covariates used in the analysis including exposure to secondhand tobacco smoke, betel nut chewing, maternal age, education level, household income level, physical activity level during pregnancy, hours spend cooking over open fire every day were captured by self-reported questionnaire.

3.2.5 Statistical Analysis

Continuous variables were assessed for normality using the Shapiro-Wilk test statistic. Toenail arsenic concentrations were right skewed and therefore transformed to their natural logarithm (ln) to improve normality of the residual in the regression model. Descriptive statistics were computed for all variables. T-test or analysis of variance was used to compare mean fetal growth factors across categories of all covariates in bivariate analysis.

We summarize our conceptual model in Figure 3-2. The exposures (A) used in the analysis were measures of maternal diet, including total energy (kcal/day), protein (gram/day), fat (gram/day), carbohydrate (gram/day), and fiber (gram/day) intakes, and the outcomes (Y) were birth outcomes, including BW (gram), GAB (week), and GWG (lb/week). Ln-transformed toenail arsenic [ln(μg/g)] was tested as the mediator (M). Simple and multiple linear regression models were used to evaluate the linear relationship between (1) A-Y, (2) M-Y, and (3) A-M, respectively. Three models were fitted for each of the relationships, including (1) a crude model, (2) an energy adjusted model where maternal intakes and toenail arsenic were adjusted for total energy intake using the residual method (56), and (3) a fully adjusted

model, where additional potential confounders (C) were added to the energy-adjusted model. To ensure comparability among effect estimates, increments of 1 s.d. were used for energy and macronutrient intakes. Potential confounders (C) controlled in the multiple linear regressions included BMI at the time of enrollment, exposure to secondhand tobacco smoke, betel nut chewing, maternal age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, hour spend cooking over open fire every day. None of the pregnant women smoked, thus, maternal smoking was not a possible confounder in this data.

We tested the mediation effect of toenail arsenic in the association of maternal diet and birth outcomes by employing a mediation analysis with counterfactual approach (Figure 3-2), using the SAS macro developed by Valeri and VanderWeele (47). The causal mediation analysis has four assumptions, including (1) no unmeasured exposure-outcome confounder conditioned on C, (2) no unmeasured mediator-outcome confounder conditioned on A and C, (3) no unmeasured exposure-mediator confounder conditioned on C, and (4) no mediator-outcome confounder affected by exposure. When these assumptions hold, the natural direct effect (NDE) represents the effect on birth outcomes if maternal intake were changed from the sample mean to mean minus 1 s.d. while keeping the toenail arsenic level for each individual at the level it would have been at intake level equals to sample mean minus 1 s.d. The natural indirect effect (NIE) represents the estimated effect on birth outcomes controlling maternal intake at the sample mean while changing the toenail arsenic level from the level it would have been at maternal intake equals to sample mean minus 1 s.d. to the level it would have been at intake level equals to sample mean. The proportion of mediation by toenail arsenic was calculated as the ratio of NIE to total effect (TE), which is the overall effect of exposure on

outcome. Two sets of mediation analysis were performed, one using maternal intake and toenail arsenic not adjusted for energy intake, and the other using energy-adjusted maternal intake and toenail arsenic concentration, both models adjusted for potential confounder mentioned above. The unadjusted model evaluated the effect of absolute exposure, while the energy-adjusted analysis evaluated the effect of proportional composition of exposure level using isocaloric comparisons. We tested for exposure-mediator interaction by running mediation analyses both with and without interaction. The presence of interaction was identified when a significant difference was observed in the effect estimates comparing models with and without interaction. We performed sensitivity analyses by stratifying by variables that may modify the effect of maternal diet and arsenic exposure on birth outcome, including drinking water arsenic level (≥50 μg/L and <50 μg/L) (53) and BMI category at enrollment. In all tests, a p-value of less than 0.05 was considered statistically significant and all tests performed were two-tailed. All statistical analyses were performed using SAS Software version 9.3 (SAS Institute, Cary, NC, USA).

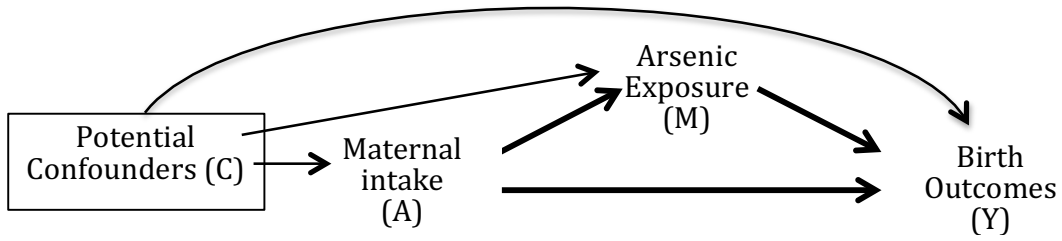

Figure 3-2. Simple conceptual model for mediation analysis in the context of the present study

3.3. Results

The average GAB in this population was 38.0 weeks (SD: 1.8 weeks; interquartile range (IQR): 31-42 weeks), the average GWG was 0.79 lb/week (SD: 0.27 lb/week; IQR:0.08-1.73 lb/weeks), and the average BW was 2843 grams (SD: 397 grams; IQR: 2610-3100 grams). The median toenail arsenic concentration was 1.21 μg/g (interquartile range: 0.65, 2.96 μg/g). Table 3-1 shows the birth outcomes according to maternal characteristics and sex of the offspring. We assessed the possible confounding effects of these characteristics on the association of maternal diet and birth outcomes. Overall, women with Cesarean births, who gave birth in hospitals, and who came from family with higher household income had higher GAB, GWG, and BW while those with higher drinking water arsenic and higher toenail arsenic, exposure to secondhand smoke had lower GAB, GWG, and BW.

Table 3-1. Characteristics of selected participants (n=1057)

Characteristics	N (%)	GAB (SD) in week	P-value	GWG (SD) in lb/week	P-value	BW (SD) in kg	p-value
Maternal age (y)							
18-20	415 (39.3)	38.06 (1.77)	0.298	0.84 (0.28)	<0.001	2833 (359)	0.662
21-25	393 (37.2)	37.89 (1.83)		0.78 (0.26)		2858 (425)	
26-41	249 (23.6)	38.08 (1.84)		0.74 (0.27)		2840 (409)	
BMI at enrollment (kg/m^2)							
<18.5	296 (28.0)	37.97 (1.77)	0.309	0.84 (0.28)	<0.001	2792 (402)	<0.001
18.5-25	663 (62.7)	37.97 (1.86)		0.79 (0.28)		2841 (379)	
25-30	88 (8.3)	38.35 (1.55)		0.69 (0.20)		3018 (463)	
>30	10 (0.9)	37.90 (1.52)		0.76 (0.23)		2997 (284)	
Infant Sex							
Male	536 (50.7)	38.02 (1.81)	0.773	0.78 (0.27)	0.214	2884 (370)	0.001
Female	521 (49.3)	37.99 (1.81)		0.80 (0.28)		2805 (417)	
Birth type							
Vaginal	692 (65.5)	37.78 (1.90)	<0.001	0.77 (0.28)	0.001	2783 (401)	<0.001
Cesarean	365 (34.5)	38.42 (1.55)		0.83 (0.27)		2959 (362)	
Birth location							
Home	581 (55.0)	37.75 (1.94)	<0.001	0.77 (0.27)	0.002	2749 (389)	<0.001
Clinic	69 (6.5)	37.81 (1.90)		0.77 (0.23)		2996 (360)	
Hospital	407 (38.5)	38.40 (1.51)		0.83 (0.28)		2964 (377)	
Drinking water arsenic (μg/L)							
<50	833 (78.8)	38.24 (1.73)	<0.001	0.81 (0.28)	<0.001	2857 (376)	0.031
≥50	224 (21.2)	37.12 (1.82)		0.72 (0.24)		2793 (463)	
Toenail arsenic (μg/g)							
0.04-0.64	270 (25.5)	38.55 (1.57)	<0.001	0.84 (0.30)	0.002	2881 (319)	0.176
0.65-1.20	259 (24.5)	37.97 (1.77)		0.80 (0.26)		2847 (375)	
1.21-2.95	263 (24.9)	37.82 (1.92)		0.77 (0.26)		2805 (450)	
2.96-46.58	265 (25.1)	37.66 (1.85)		0.76 (0.26)		2842 (428)	
Secondhand tobacco smoke							
No	613 (58.0)	38.05 (1.81)	0.298	0.81 (0.28)	0.010	2874 (393)	0.003
Yes	444 (42.0)	37.93 (1.82)		0.77 (0.26)		3801 (398)	
Betel Nut chewing							
No	1046 (99.0)	38.00 (1.81)	0.244	0.79 (0.27)	0.642	2845 (397)	0.254
Yes	11 (1.0)	38.64 (1.75)		0.83 (0.29)		2708 (321)	
Maternal education							
Secondary	562 (53.2)	37.84 (1.79)	<0.001	0.81 (0.27)	0.025	2729 (381)	0.001
Primary	342 (32.4)	38.37 (1.73)		0.79 (0.28)		2862 (380)	
Illiterate	153 (14.5)	37.78 (1.92)		0.75 (0.27)		2864 (405)	
Household monthly income							
<25 USD	10 (0.9)	38.30 (2.08)	0.001	0.86 (0.30)	<0.001	2624 (336)	0.002
25-36 USD	170 (16.1)	37.64 (1.87)		0.71 (0.27)		2818 (402)	
37-49 USD	287 (27.2)	37.78 (1.89)		0.77 (0.24)		2837 (393)	
50-61 USD	308 (29.1)	38.12 (1.77)		0.80 (0.28)		2807 (423)	
62-74 USD	157 (14.9)	38.23 (1.72)		0.82 (0.29)		2877 (358)	
>75 USD	125 (11.8)	38.39 (1.60)		0.88 (0.28)		2962 (354)	
Pregnancy activity level							
Low	55 (5.2)	37.76 (2.08)	0.489	0.80 (0.25)	0.960	2764 (581)	0.005
Medium	950 (89.9)	38.02 (1.78)		0.79 (0.28)		2862 (382)	
High	32 (3.0)	37.81 (2.12)		0.79 (0.26)		2718 (399)	
Hours spent cooking over open fire during pregnancy (hr/day)							
0-2 hr	79 (7.5)	38.65 (1.64)	0.002	0.88 (0.28)	0.017	2894 (310)	0.281
2-4 hr	531 (50.2)	37.88 (1.88)		0.78 (0.27)		2827 (414)	
4-6 hr	447 (42.3)	38.04 (1.73)		0.79 (0.27)		2854 (388)	

In Table 3-2, we present mean difference in GAB, GWG, and BW by quartiles of total energy and macronutrient intakes comparing each quartile to the first (lowest) quartile as the reference. P-value for trend derived from univariate regression showed no significant trend in GAB, GWG, and BW by quartile increase in total energy intake. However, GAB, GWG, and BW all decreased across increasing quartiles of protein, fiber and fat intakes, and in contrast, increased across quartile of carbohydrate intakes (all p-for-trend <0.001).

Table 3-2. Mean change and standard error (S.E) in birth outcomes by quartile of energy and macronutrient intakes (n=1057)

	Q1	Q2	Q3	Q4	p-for-trend
GAB (week)					
Energy	Ref	-0.378 (0.155)	-0.660 (0.155)	0.125 (0.148)	0.458
Protein	Ref	-0.176 (0.117)	-1.460 (0.145)	-1.989 (0.140)	<0.001
Fat	Ref	-0.279 (0.124)	-1.360 (0.144)	-1.698 (0.142)	<0.001
Carbohydrate	Ref	0.456 (0.161)	1.630 (0.140)	1.909 (0.136)	<0.001
Fiber	Ref	-0.241 (0.137)	-1.447 (0.149)	-1.409 (0.145)	<0.001
GWG (lb/week)					
Energy	Ref	-0.002 (0.024)	-0.031(0.023)	0.013 (0.024)	0.892
Protein	Ref	-0.008 (0.024)	-0.110 (0.024)	-0.159 (0.022)	<0.001
Fat	Ref	-0.010 (0.024)	-0.062 (0.024)	-0.122 (0.022)	<0.001
Carbohydrate	Ref	0.060 (0.022)	0.167 (0.023)	0.135 (0.021)	<0.001
Fiber	Ref	-0.005 (0.024)	-0.100 (0.023)	-0.136 (0.023)	<0.001
BW (gram)					
Energy	Ref	-9.05 (34.80)	-15.01 (34.79)	77.66 (33.74)	0.037
Protein	Ref	-26.38 (25.88)	-171.21 (34.69)	-128.95 (34.20)	<0.001
Fat	Ref	-62.28 (29.52)	-150.15 (32.48)	-94.91 (36.58)	<0.001
Carbohydrate	Ref	79.83 (39.90)	56.26 (34.41)	128.21 (34.31)	<0.001
Fiber	Ref	-20.79 (29.11)	-150.75 (33.08)	-48.33 (35.54)	<0.001

[1]mean energy intake 3059.3±709.2 kcal/day, mean adjusted protein intake 167.4±55.4 g/day, mean adjusted fat intake 63.2±14.4 g/day, mean adjusted carbohydrate intake 437.9±76.2 g/day, mean adjusted fiber intake 44.8±8.5 g/day. Intakes adjusted for total energy intake using the residual method.

3.3.1 Associations between exposures and outcomes

Table 3-3 showed the associations between maternal diet and birth outcomes. Total energy was associated with GAB (fully adjusted β =0.114, 95% CI=0.006-0.222) , but not with GWG (fully adjusted β=0.002, 95% CI=-0.014-0.018) or BW (fully adjusted β=14.2, 95% CI=-8.9 - 37.3). All macronutrients intakes were significantly associated with GAB and GWG. Similar negative direction of association was observed for protein, fat and fiber intakes, while carbohydrate displayed an opposite positive direction of association. The effect estimates increased in strength after adjusting for total energy intake and after controlling for other potential confounders, the significant associations remained for the relationship between all maternal intake and GAB and GWG. For associations between maternal intake and BW, only the effect estimate for protein intake remained significant after adjusting for energy and potential confounders, specially, each s.d. increase (55.6 gram/day) in maternal protein intake was associated with 32.3 grams (95% CI: 4.5-60.1) lower birth weight infants.

Table 3-3. Partial regression coefficient showing change in birth outcomes per increment of 1 s.d.[1] of total energy and macronutrient intakes (n=1057)

	Crude[2]		Adjust for energy[3]		Fully Adjusted[4]	
	$\hat{\beta}$(SE)	P-value	$\hat{\beta}$(SE)	P-value	$\hat{\beta}$(SE)	P-value
GAB (week)						
Energy	0.120 (0.056)	0.031	-	-	0.114 (0.055)	0.040
Protein	-0.707 (0.051)	<0.001	-0.828 (0.050)	<0.001	-0.870 (0.060)	<0.001
Fat	-0.456 (0.005)	<0.001	-0.650 (0.052)	<0.001	-0.575 (0.057)	<0.001
Carbohydrate	0.572 (0.053)	<0.001	0.791 (0.050)	<0.001	0.789 (0.060)	<0.001
Fiber	-0.355 (0.055)	<0.001	-0.586 (0.053)	<0.001	-0.487 (0.060)	<0.001
GWG (lb/week)						
Energy	0.002 (0.008)	0.779	-	-	0.002 (0.008)	0.773
Protein	-0.059 (0.008)	<0.001	-0.066 (0.008)	<0.001	-0.061 (0.010)	<0.001
Fat	-0.036 (0.008)	<0.001	-0.046 (0.008)	<0.001	-0.035 (0.009)	<0.001
Carbohydrate	0.038 (0.008)	<0.001	0.061 (0.008)	<0.001	0.053 (0.010)	<0.001
Fiber	-0.038 (0.008)	<0.001	-0.053 (0.008)	<0.001	-0.046 (0.009)	<0.001
BW (gram)						
Energy	19.1 (12.2)	0.117	-	-	14.2 (11.8)	0.231
Protein	-46.0 (33.4)	<0.001	-58.7 (12.1)	<0.001	-32.3 (14.2)	0.023
Fat	-14.8 (12.2)	0.226	-32.3 (12.2)	0.008	-11.1 (12.9)	0.389
Carbohydrate	43.5 (12.1)	<0.001	46.9 (12.1)	<0.001	20.2 (13.8)	0.144
Fiber	-1.45 (12.2)	0.905	-19.5 (12.2)	0.110	7.6 (13.2)	0.565

[1] s.d. for energy 606.9 kcal/day, for protein 55.6 g/day, for fat 14.2 g/day, for carbohydrates 76.1 g/day, for fibers 8.4 g/day.
[2] crude model using unadjusted intake level
[3] intake level adjusted for energy using the residual method
[4] intake level adjusted for energy using residual method (except for energy), regression model adjusted for BMI at the time of enrollment, exposure to secondhand tobacco smoke, betel nut chewing, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, daily hours spent cooking over an open fire

3.3.2 Associations between exposures and mediator

Table 3-4 showed the partial regression coefficient of models assessing the association of maternal intakes with ln-transformed toenail arsenic. Total energy intake was not significantly associated with ln-transformed toenail arsenic (fully adjusted model, p=0.77). Protein, fat and fiber intake were positively associated with ln-transformed toenail arsenic while carbohydrate intake was negatively associated with ln-transformed toenail arsenic. Energy adjustment increased the absolute effect estimates for all association between macronutrient intakes and toenail arsenic. The significant associations remained after adjusting potential confounders though with minor attenuations. Specifically, each s.d. increase in protein, fat, and fiber intake were associated with 0.33 (95% CI: 0.26, 0.41), 0.23 (95% CI: 0.16, 0.30), and 0.26 (95% CI: 0.19, 0.33) ln(μg/g) increase in ln-transformed toenail arsenic, while each s.d. increase in carbohydrate intake was associated with 0.32 (95% CI: -0.39, -0.25) decrease in ln-transformed toenail arsenic.

Table 3-4. Partial regression coefficients showing changes in toenail arsenic concentration per increment of 1 s.d. of total energy and macronutrient intake[1]

	Crude[3]		Adjust for energy[4]		Fully Adjusted[5]	
Intakes	$\hat{\beta}$(SE)	P-value	$\hat{\beta}$(SE)	P-value	$\hat{\beta}$(SE)	P-value
Energy	0.002 (0.033)	0.958	-	-	-0.010 (0.033)	0.770
Protein	0.374 (0.031)	<0.001	0.410 (0.031)	<0.001	0.331 (0.038)	<0.001
Fat	0.266 (0.032)	<0.001	0.326 (0.032)	<0.001	0.233 (0.035)	<0.001
Carbohydrate	-0.240 (0.033)	<0.001	-0.402 (0.031)	<0.001	-0.320 (0.037)	<0.001
Fiber	0.261 (0.032)	<0.001	0.348 (0.031)	<0.001	0.257 (0.036)	<0.001

[1] s.d. for energy 606.9 kcal/day, for protein 55.6 g/day, for fat 14.2 g/day, for carbohydrates 76.1 g/day, for fibers 8.4 g/day.
[2] ln-transformed toenail arsenic concentration, unit (ln(μg/g)).
[3] crude model
[4] intake level and ln-transformed toenail arsenic concentration adjusted for energy using the residual method (except for energy)
[5] intake level and ln-transformed toenail arsenic concentration adjusted for energy using residual method (except for energy), regression model adjusted for BMI at the time of enrollment, exposure to secondhand tobacco smoke, betel nut chewing, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, daily hours spent cooking over an open fire

3.3.3 Association between mediator and outcomes

Table 3-5 showed the partial regression coefficient of the models assessing the association of ln-transformed toenail arsenic with birth outcomes. Similar to previous findings (30), higher toenail arsenic concentration was associated with lower GAB and lower GWG, and the effect remained significant after adjusting for total energy and potential confounders. Specifically, the fully adjusted model showed that each unit increase in ln-transformed toenail arsenic (ln(μg/g)) was associated with a decrease of 0.17 week (95% CI: -0.28, -0.07) in GAB or a decrease of 0.02 lb/week (95% CI: -0.04, -0.01) in GWG. Higher toenail arsenic was associated with lower BW, however, the association was not statistically significant.

Table 3-5. Partial regression coefficient showing changes in birth outcomes per unit increase toenail arsenic[1] (*n*=1057)

	Crude[2]		Adjust for energy[3]		Fully Adjusted[4]	
Outcome	$\hat{\beta}$(SE)	P-value	$\hat{\beta}$(SE)	P-value	$\hat{\beta}$(SE)	P-value
GAB (week)	-0.276 (0.051)	<0.001	-0.277 (0.051)	<0.001	-0.173 (0.052)	0.001
GWG (lb/week)	-0.029 (0.008)	<0.001	-0.029 (0.008)	<0.001	-0.020 (0.008)	0.011
BW (gram)	-13.96 (11.23)	0.214	-14.20 (11.23)	0.206	-5.62 (11.3)	0.618

[1] ln-transformed toenail arsenic concentration (ln(μg/g)).
[2] crude model
[3] ln-transformed toenail arsenic adjusted for energy intake using residual method
[4] using ln-transformed toenail arsenic adjusted for energy intake using residual method and regression model adjusted for BMI at the time of enrollment, exposure to secondhand tobacco smoke, betel nut chewing, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, daily hours spent cooking over an open fire every day

3.3.4 Mediating effect of toenail arsenic concentration in the relationship between maternal diet with birth outcomes

The associations between diet, ln-transformed toenail arsenic and birth outcomes are summarized by graphic illustration in Figure S3-1 to S3-3. Mediation analyses were performed only on the pathways with significant exposure-mediator and exposure-outcome relationships. The exposure-mediator interaction was explored by including an exposure-mediator interaction term in the multiple linear regression models, significant interaction with toenail arsenic was observed for total energy but not for any of the macronutrients in the associations with birth outcomes. In the analysis with total energy intake, we observed significant association only with GAB but not with BW or GWG. Therefore, mediation analysis accounting for interaction was only performed on the association between total energy intake and GAB. Controlling for toenail arsenic level and all other potential confounders, total energy intake had significant NDE and TE on GAB, each s.d. increase from the mean in total energy intake was associated with 0.112 (95% CI: 0.002, 0.221) week increase in GAB while holding the toenail arsenic at

the level it would have naturally been at the population mean (Table 3-6).

In the analysis without energy adjustment, there were significant NDEs and TE by all absolute macronutrients intakes on GAB and GWG but not BW (Table 3-6). The directions of association were consistent with those found in the linear regression analyses. Significant NIEs by toenail arsenic was observed for the associations between absolute fat, carbohydrate, and fiber intakes on GAB. The data suggested that 6% of the association between absolute fat intake and GAB may be mediated by difference in toenail arsenic level; while the corresponding percent mediated was 3% for carbohydrate and 10% for fiber intake.

In the analysis with total energy adjustment, increased strength of association in NDEs and TEs were observed between all macronutrients with GAB and GWG as compared to the analysis without energy adjustment, however, the NIEs by toenail arsenic were not statistically significant (Table 3-7). In the analysis on BW, we only observed significant association with protein intake on BW, and significant NDE and TEs were observed in analysis controlling for total energy intakes.

Table 3-6. Mediation analysis of the estimated effect[1] (95% CI) of maternal energy and nutrient intake (per s.d. increment)[2] on birth outcomes through toenail arsenic[2] (ln(μg/g)) (n=1057), with no energy adjustment

	Natural direct effect (95% CI)	Natural indirect effect (95% CI)	Total effect (95% CI)	Percent Mediated (%)[3]
GAB (week)				
Energy[4]	**0.112 (0.002, 0.221)**	0.002 (-0.009,0.013)	**0.113 (0.003, 0.224)**	
Protein	**-0.664 (-0.786, -0.542)**	-0.013 (-0.041, 0.016)	**-0.676 (-0.795, -0.558)**	-
Fat	**-0.339 (-0.452, -0.226)**	**-0.022 (-0.042, -0.001)**	**-0.362 (-0.473, -0.250)**	6% (1%-9%)
Carbohydrate	**0.471 (0.359, 0.582)**	**0.017 (0.001,0.034)**	**0.488 (0.377, 0.599)**	3% (1%-6%)
Fiber	**-0.216 (-0.331, -0.101)**	**-0.025 (-0.046, -0.005)**	**-0.241 (-0.355, -0.127)**	10% (4%-13%)
GWG (lb/week)				
Protein	**-0.047 (-0.066, -0.028)**	-0.003 (-0.008, 0.001)	**-0.050 (-0.069, -0.031)**	-
Fat	**-0.021 (-0.044, -0.040)**	-0.003 (-0.007, 0.000)	**-0.025 (-0.042, -0.008)**	12% (0%-17%)
Carbohydrate	**0.026 (0.009, 0.043)**	0.003 (-0.001, 0.005)	**0.028 (0.011, 0.045)**	-
Fiber	**-0.026 (-0.043, -0.009)**	-0.003 (-0.006, 0.000)	**-0.029 (-0.046, -0.012)**	10% (0%-13%)
BW (gram)				
Protein	-22.82 (-50.39, 4.75)	-2.90 (-3.59, 9.39)	-19.92 (-46.74, 6.90)	**-**
Carbohydrate	23.89 (-0.786, 48.57)	-1.33 (-4.76, 2.10)	22.56 (-1.89, 47.01)	**-**

Abbreviations: CI, confidence interval. Bold letter indicates p<0.01

[1] The natural direct effect, natural indirect effect, and total effects reflect the change in gestational week (week), gestational weight gain rate (lb/week), or birth weight (gram) per s.d. increase in intake and are measured based on intake change from mean minus 1 s.d to mean. Model was adjusted for BMI at the time of enrollment, exposure to environmental tobacco smoke, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, and daily hours spent cooking over an open fireevery day.

[2] absolute intake and unadjusted toenail arsenic concentration

[3] Percent mediated = (Natural indirect effect/total effect)*100%

[4] Controlled for exposure-mediator interaction.

Table 3-7. Mediation analysis of the estimated effect1 (95% CI) of maternal energy and nutrient intake (per s.d. increment)[2] on birth outcomes through toenail arsenic[2] (ln(μg/g)) (n=1057), with energy adjustment using the residual method

Intake	Natural direct effect (95% CI)	Natural indirect effect (95% CI)	Total effect (95% CI)	Percent Mediated (%)[3]
GAB (week)				
Protein	**-0.873 (-0.996, -0.750)**	0.003 (-0.030, 0.035)	**-0.870 (-0.989, -0.751)**	-
Fat	**-0.558 (-0.673, -0.443)**	-0.017 (-0.041, 0.007)	**-0.575 (-0.687, -0.462)**	-
Carbohydrate	**0.787 (0.665, 0.908)**	0.002 (-0.030, 0.034)	**0.789 (0.671, 0.906)**	-
Fiber	**-0.465 (-0.586, -0.645)**	-0.022 (-0.049, 0.005)	**-0.488 (-0.605, -0.370)**	-
GWG (lb/week)				
Protein	**-0.058 (-0.078, -0.038)**	-0.003 (-0.008, 0.003)	**-0.061 (-0.080, -0.041)**	-
Fat	**-0.031 (-0.049, -0.013)**	-0.003 (-0.007, 0.001)	**-0.035 (-0.052, -0.017)**	-
Carbohydrate	**0.050 (0.031, 0.069)**	0.003 (-0.002, 0.008)	**0.053 (0.034, 0.071)**	-
Fiber	**-0.042 (-0.061, -0.024)**	-0.003 (-0.007, 0.001)	**-0.046 (-0.064, -0.028)**	-
BW (gram)				
Protein	**-36.74 (-65.5, -7.95)**	4.40 (-3.23, 12.02)	**-32.34 (-60.14, -4.54)**	-
Carbohydrate	23.59 (-4.46, 51.54)	-3.40 (-10.75, 3.95)	20.19 (-6.91, 47.28)	-

Abbreviations: CI, confidence interval. Bold letter indicates $p<0.01$

[1] The natural direct effect, natural indirect effect, and total effects reflect the change in gestational week (week) gestational weight gain rate (lb/week), or birth weight (gram) per s.d increase in intake and are measured based on intake change from mean minus 1 s.d to mean. Model was adjusted for BMI at the time of enrollment, exposure to environmental tobacco smoke, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, and daily hours spent cooking over an open fire every day.

[2] intake level and toenail arsenic level adjusted for energy using the residual method (except for energy)

[3] Percent mediated = (Natural indirect effect/total effect)*100%

3.3.5 Power and Sensitivity Analysis

Using the full cohort (N=1057), all mediation analyses had more than 95% power to detect mediation effect controlling type I error α=0.05. Due to the strong collinearity between drinking water arsenic and toenail arsenic (Spearman ρ=0.51), we did not adjust for drinking water arsenic in the mediation analysis. We conducted sensitivity analyses by stratifying subjects by drinking water arsenic concentration (Supplementary Table S3-1 to S3-6). The reported association remained when restricting the analysis to those exposed to water arsenic <50 μg/L (n=833), but not for those with water arsenic level arsenic ≥50 μg/L (n=224), possibly due to lack of power (power=11~22%). After stratifying by water arsenic level, the significant mediation effects by toenail arsenic no longer hold, likely due to the reduced variation in toenail arsenic level. Similar results were found for all birth outcomes and analyses with and without energy-adjustment.

The second sensitivity analysis was performed stratifying by normal or underweight BMI; similarly, the significant indirect effect mediated by toenail arsenic concentration was no longer observed, possibly due to reduced variation in toenail arsenic level. Among pregnant women with normal BMI (n=751), the reported effects remained consistent for most associations between maternal diet and birth outcomes, except that the effect by total energy intake on GAB became nonsignficant (Supplementary Table S3-1). The NIE from toenail arsenic concentration on GAB attenuated to null among those exposed to water arsenic level <50 μg/L. Analyses restricting to underweight women (BMI <18.5 kg/m^2) had higher strength of association in general, suggesting the effect of change in diet intake had stronger effect on birth outcomes among women with underweight BMI.

3.4. Discussion

To our knowledge, this is the first study to use epidemiological data to study the mediating effect of long-term arsenic exposure in the relationship of maternal diet on birth outcomes factors relating to BW. Using a prospective birth cohort in Bangladesh where the water arsenic level ranges widely, we were able to assess the effect of long-term arsenic exposure on pregnancy outcome. We employed a validated semi-quantitative FFQ (53) to capture long-term maternal diet habit during pregnancy and used a causal mediation analysis to investigated the effect of arsenic exposure on relationship of maternal diet with BW.

Mediation analysis is an established method used in social and epidemiological studies to understand the impact of variables in causal pathways or biological mechanisms. Recently, the concept of counterfactual framework (57) has been incorporated into mediation analysis to for a more precise definition of the necessary assumption as well as for extensions to more complex model that enables exposure-mediator interaction (47). The application of this causal mediation in environmental health research allows researchers to gain qualitative prospective on the NDE by exposure and the NIE by mediator while testing for interaction between exposure and mediator. The mediation analyses showed that 6% (95% CI: 1%-6%) and 10% (95% CI: 4%-13%) of the decreases in GAB resulted from increasing absolute fat and fiber intakes, respectively, from mean minus 1 s.d. to the mean was mediated by increase arsenic exposure level associated with the change in intake. The positive associations between fat and fiber consumptions with elevated arsenic exposure can be supported by evidence from other studies; finding from the same birth cohort indicated consumptions of fish, meat and vegetable dishes were associated with higher toenail arsenic (46). A positive association of dark meat consumption with toenail arsenic was also found in a cohort in New Hampshire(58). Elevated

level of arsenic has been found in vegetables grown in Bangladesh (59,60) and a food survey in Pabna, Bangladesh which collected 6 days of duplicated food samples from 47 families showed that the median daily total As intake was 48 μg As/day from food (61). In contrast, the negative association between carbohydrate intake and arsenic exposure level, which drives 3% of the increase in GAB while increasing the carbohydrate intake level from the mean minus 1 s.d. to the mean, is less clearly understood. Carbohydrate intake was mainly contributed by intakes of grain, cereal, and bread. While rice has been known to accumulate inorganic arsenic (60,62), in our cohort we did not observe a significant association between rice consumption and elevated toenail arsenic level (46). Data from our cohort did indicate that intakes of other carbohydrate rich food, including fried bread, rice cereal and homemade snacks, were negatively associated with toenail arsenic concentration(46). These negative associations could indicate replacement of rice consumption with other carbohydrate source that had lower arsenic level, or other factors which led to overall lower cumulative arsenic exposure level.

One of the pathways (37) by which nutrition and environmental exposures may interact is the increase of overall exposure level and body burden from food intake. Diet may be a contributor to arsenic exposure. our data indicated a mediating effect of cumulative arsenic level in the toenail in the association between maternal diet and birth outcomes, although the absolute magnitude of mediation was small. This mediating effect by arsenic were observed only when looking at absolute intake of nutrients and arsenic but not at energy-adjusted intake, suggesting that the mediating effect of arsenic exposure was mainly driven by the quantity of intake rather than the intake in proportion to other dietary components.

The reason we did not observea significant mediating effect when controlling for total energy intake could be two-fold. First, the energy-adjusted mediating effect by arsenic

exposure might be too small and the variation of macronutrient intake in proportion to total energy in our study population was not wide enough to observe a significant mediating effect. Second, there may be other potential causal pathways beyond mediation by arsenic by which calorie-adjusted macronutrient intake and arsenic exposure may influence GAB and GWG. Examples of the potential mediators are factors that may influence one's susceptibility to arsenic toxicity, such as arsenic bioavailability and arsenic methylation capacity. Specifically, new evidence suggests that diet with a high proportion of energy from fat and protein may reduce gastrointestinal bioacessbility of arsenic but may increase arsenic speciation changes in the colon influencing arsenic toxicity (63). Arsenic methylation capacity, which is generally considered as ones' ability to methylate, detoxify and eliminate arsenic from the body, may be influenced by various factors, including dietary pattern and nutritional status (64). Lower protein intake level had found to be associated with poorer arsenic methylation capacity as compared to those with higher protein intake (65), and consumption of seafood, seaweed and rice which has high arsenic contents may also interfere with the arsenic methylation profile (64). These are factors may be changed by maternal dietary intake and alter birth outcomes. Future research to test the mediating effect of these variables can help elucidate the complex relationship between maternal diet, arsenic exposure and birth outcome.

In the association between maternal diet and birth outcome, we did observe a significant positive association of energy intake with GAB. We did not however observe an association of total energy intake with GWG nor BW, in contrast to findings from some prior studies (66–72). Other studies (73–77), however, also reported no consistent association, suggesting the relationship between energy intake and GWG and BW is complex and may be influenced by many other socioeconomic, cultural, and environmental factors unaccounted for in these

studies. Lechtig and et al. suggested that the effect of increase total caloric intake has a threshold effect, where a minimal level of nutrients must be available to achieve adequate birth weight, however, above the minimal threshold, the wide variety of maternal intake, both in quantity and quality, may not have significant effect on birth weight (34). Susser also pointed out that the causal sequence of maternal diet, maternal weight gain and infant birth weight could only be demonstrated below a famine threshold (78). In our population, all pregnant women received prenatal medical care and prenatal multivitamin upon enrollment into the study; therefore, the problem of severe malnutrition was not likely, making the direct association between maternal total caloric intake and BW unlikely to be observed.

When examining the effects of individual macronutrient intakes, we found positive associations for absolute and energy-adjusted carbohydrate intakes with GAB and GWG. Carbohydrates provided a substantial proportion of the energy in the diet of our study population. Common sources of carbohydrate in the diet were rice, bread, and rice cereal. In a systematic review on the maternal macronutrient intake and gestational weight gain, three out of 18 observations studies, which took place in developed settings, reported higher GWG associated with increased carbohydrate intake while the other 15 studies did not yield consistent results (72). Our data showed that greater protein and fat intake were associated with lower GAB and GWG. These negative associations had also been reported other observational studies for protein intake but not for fat intake (68,71,72,79). Higher protein and fat intake may lead to a deviation from optional dietary composition for fetal growth. High protein diet had been shown to cause harmful effect to pregnancy where pooled data from three clinical trials with isocaloric protein supplementation found increased risks for small-for-gestational age birth (RR=1.35, 95% CI=1.12-1.16) (80,81). Higher consumption of protein may decrease

GWG through the mechanism of requiring higher energy expenditure since the thermogenesis of protein is higher than that of carbohydrate (82, 83). The negative association could also indicate diet that is associated with increased exposure to other reproductive toxins and heavy metals. Turmeric (82) and vegetables (59) in Bangladesh have been found to contain high level of lead; elevated exposure of mercury can come from higher consumption of fish (83). Poultry meat, fish, vegetables obtained from area polluted by the tannery industry in Dhaka, Bangladesh, which is about 20 km away from one of our study site, showed unsafe level of heavy metal contaminations with chromium, lead, and mercury (84). Greater fat intake may also increase the absorption endocrine disruption chemicals, which are generally lipophilic, increasing body's burden to environmental pollutants. Last but not least, higher consumption of protein, fat and fiber may also increase satiety, which may affect the total energy intake and dietary composition. Greater fiber consumption may also impair the absorption of minerals, including iron, zinc, magnesium, calcium and phosphorus (85,86).

Our study had several strengths. First of all, the potential measurement error for the mediator is likely to be minimal and nondifferential since the analysis of toenail arsenic was independent of subject collection and the research technician was blinded to subjects' information, making the error of toenail arsenic measurement independent of the outcome, exposure and the covariates. It has been demonstrated that when the mediator is continuous and the measurement error is nondifferentially misclassified, the ordinary least squares (OLS) estimator of the coefficient of the exposure-mediator regression are asymptotically unbiased (87). Second, DCH was the primary healthcare provider in our study area; thus, all the women received the same level of prenatal care minimizing the potential unmeasured confounding related to prenatal care. Third, we were able to control for many important

potential confounders, including physical activity level during pregnancy, maternal BMI at enrollment and maternal age at the time of pregnancy to increase the validity of our estimate. However, we recognized that unmeasured confounding variable might exist, such as food quality and inter-pregnancy interval since that information were not available in the cohort.

A limitation of our study is that FFQ is not an instrument intend to capture the exact level of intake. The average energy and macronutrient intake level reported by the women in our cohort were higher than the recommended intake level for pregnant women and national average reported in the Bangladesh Household and Income Survey (88). We attempted to minimize this problem of overestimation by standardizing each intake level with its standard deviation. The problem of overestimation would not affect the validity of the FFQ to rank relative intake levels, which the FFQ had been tested to have sufficient statistical power to do so (53); thus, provided the limited resource available to conduct dietary assessment in a large prospective study, using the FFQ was the best option that still allowed a good comparison of dietary intake level across study subjects. A validation study of the FFQ using residents in Pabna, Bangaldesh showed that carbohydrate intake measured by this FFQ may exhibit negative proportional bias as intake level increases, meaning, as the intake level increases, carbohydrate intake tended to be lower compared to true intake (53). Under this circumstance, a weaker effect of association would be observed since the underestimation would bias the effect toward the null. Using the FFQ also had the disadvantage of recall bias, where participants tend to report the most recent intake (89). We conducted analyses using intake data reported by the pregnant women at 28 weeks of gestation and found similar results (data not shown), suggesting the dietary pattern in our study population was quite consistent and the problem of recall bias should not affect the validity of the result. Using ultrasound-calculated

GAB may introduce a potential source of bias. Ultrasound is the gold standard method to estimate GA at early pregnancy, but the estimation may not be as accurate at the second trimester (90). In a previous published study from the same cohort (30), we discussed the potential bias that may be introduced if GA at enrollment was associated with arsenic exposure. After careful examinations, the potential for bias was concluded to be small, as no correlation was found between GA at enrollment and ln-transformed toenail arsenic and GA at enrollment was not significantly different between women with different water arsenic exposure level (above or below the Bangladesh national drinking water standard of 50 μg/L) (30). Another limitation of our study was that we were not able to examine the diet quality. We tried to control for factors that may influence diet quality in Bangladesh, such as educational level and family income level (91), to account for the variation that may be introduced by diet quality, however, that might not be an accurate estimate of dietary quality. Observational and interventional studies had reported higher diet quality was associated with higher birth weight (92,93), reduced gestational hypertension(94), lower risk of gestational diabetes(95), and lower prevalence of low birth weight (96). While poor diet quality may increase neonatal adiposity (97). However, some studies indicated no significant association between diet quality with GWG (98,99). Diet quality can be assessed by indicators such as the USDA Healthy Eating Index scale and the Healthy Food Intake Index (HFII) (100–102). Such scale had not been adopted in Bangladesh (103), so it was not possible to examined the quality of the diet in our study. The diet quality in Bangladesh has been assessed using diet diversity score calculated based on 24-hour diet recall and found that the diet quality of pregnant women was poor and intake of micronutrient-rich food to be low (104). Developing a method to assess diet quality can be the future direction of our research. Strategy of using FFQ to calculate food quality

score, such as the one used in HFII (102), can be adopted to estimate the diet quality in our study population.

3.5. Conclusions

In our study population, maternal energy and macronutrient intakes were significantly associated with GAB and GWG, and not more than 10% of the effect of each macronutrient was mediated via toenail arsenic level. After adjusting for total energy, no significant mediating effect was found, suggesting the absolute amount of arsenic exposure rather than the arsenic level in relationship to total energy intake was a more important factor to consider when understanding the negative implication of arsenic on birth outcomes.

3.6. References

1. Agency for Toxic Substance and Disease Registry (ATSDR). ATSDR, Agency for Toxic Substances and Disease Registry: Toxicological Profile for Arsenic. Atlanta, GA, USA; 2000.
2. WHO. Exposure to arsenic: A major public health concern [Internet]. 2010 [cited 2015 Apr 15]. Available from: http://www.who.int/ipcs/assessment/public_health/arsenic/en/
3. Smedley P., Kinniburgh D. A review of the source, behaviour and distribution of arsenic in natural waters. Appl Geochemistry. 2002;17:517–68.
4. Smith AH, Lingas EO, Rahman M. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. Bull World Health Organ. 2000;78:1093–103.
5. Kinniburgh D, Smedley P. Arsenic contamination of groundwater in Bangladesh.British Geological Survey Technical Report WC/00/19. Keyworth; 2001.
6. Kurzius-Spencer M, Burgess JL, Harris RB, Hartz V, Roberge J, Huang S, Hsu C-H, O'Rourke MK. Contribution of diet to aggregate arsenic exposures-an analysis across populations. J Expo Sci Environ Epidemiol. NIH Public Access; 2014;24:156–62.
7. Carlin DJ, Naujokas MF, Bradham KD, Cowden J, Heacock M, Henry HF, Lee JS, Thomas DJ, Thompson C, Tokar EJ, et al. Arsenic and Environmental Health: State of the Science and Future Research Opportunities. Environ Health Perspect. National Institute of Environmental Health Science; 2016;124:890–9.
8. Dabeka RW, McKenzie AD, Lacroix GM, Cleroux C, Bowe S, Graham RA, Conacher HB, Verdier P. Survey of arsenic in total diet food composites and estimation of the dietary intake of arsenic by Canadian adults and children. J AOAC Int. 1993;76:14–25.
9. MacIntosh DL, Spengler JD, Ozkaynak H, Tsai L, Ryan PB. Dietary exposures to

selected metals and pesticides. Environ Health Perspect. 1996;104:202–9.

10. Meharg AA, Rahman MM. Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. Environ Sci Technol. 2003;37:229–34.

11. Duxbury JM, Mayer AB, Lauren JG, Hassan N. Food chain aspects of arsenic contamination in Bangladesh: effects on quality and productivity of rice. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2003;38:61–9.

12. Pizarro I, Gómez MM, Fodor P, Palacios MA, Cámara C. Distribution and biotransformation of arsenic species in chicken cardiac and muscle tissues. Biol Trace Elem Res. 2004;99:129–43.

13. Schmidt CW. Arsenical Association: Inorganic Arsenic May Accumulate in the Meat of Treated Chickens. Environ Health Perspect. 2013;121:a226–a226.

14. Cubadda F, Jackson BP, Cottingham KL, Van Horne YO, Kurzius-Spencer M. Human exposure to dietary inorganic arsenic and other arsenic species: State of knowledge, gaps and uncertainties. Sci Total Environ. 2017;579:1228–39.

15. World Health Organization W. Evaluations of Certain Contaminants in Food: seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva, Switzerland; 2011.

16. Smith AH, Hopenhayn-Rich C, Bates MN, Goeden HM, Hertz-Picciotto I, Duggan HM, Wood R, Kosnett MJ, Smith MT. Cancer risks from arsenic in drinking water. Environ Health Perspect. 1992;97:259–67.

17. Chen C-L, Hsu L-I, Chiou H-Y, Hsueh Y-M, Chen S-Y, Wu M-M, Chen C-J. Ingested arsenic, cigarette smoking, and lung cancer risk: a follow-up study in arseniasis-endemic

areas in Taiwan. JAMA. 2004;292:2984–90.

18. Marshall G, Ferreccio C, Yuan Y, Bates MN, Steinmaus C, Selvin S, Liaw J, Smith AH. Fifty-Year Study of Lung and Bladder Cancer Mortality in Chile Related to Arsenic in Drinking Water. JNCI J Natl Cancer Inst. 2007;99:920–8.

19. Wang W, Cheng S, Zhang D. Association of inorganic arsenic exposure with liver cancer mortality: A meta-analysis. Environ Res. 2014;135:120–5.

20. Saint-Jacques N, Parker L, Brown P, Dummer TJ. Arsenic in drinking water and urinary tract cancers: a systematic review of 30 years of epidemiological evidence. Environ Health. 2014;13:44.

21. Vahter ME. Interactions between Arsenic-Induced Toxicity and Nutrition in Early Life. J Nutr. 2007;137:2798–804.

22. Tseng WP. Effects and dose--response relationships of skin cancer and blackfoot disease with arsenic. Environ Health Perspect. 1977;19:109–19.

23. Ahsan H, Chen Y, Parvez F, Zablotska L, Argos M, Hussain I, Momotaj H, Levy D, Cheng Z, Slavkovich V, et al. Arsenic exposure from drinking water and risk of premalignant skin lesions in Bangladesh: baseline results from the Health Effects of Arsenic Longitudinal Study. Am J Epidemiol. 2006;163:1138–48.

24. Maull EA, Ahsan H, Edwards J, Longnecker MP, Navas-Acien A, Pi J, Silbergeld EK, Styblo M, Tseng C-H, Thayer KA, et al. Evaluation of the Association between Arsenic and Diabetes: A National Toxicology Program Workshop Review. Environ Health Perspect. 2012;

25. Quansah R, Armah FA, Essumang DK, Luginaah I, Clarke E, Marfo K, Cobbina SJ, Nketiah-Amponsah E, Namujju PB, Obiri S, et al. Association of Arsenic with Adverse

Pregnancy Outcomes-Infant Mortality: A Systematic Review and Meta-Analysis. Environ Health Perspect. 2015;

26. Tofail F, Vahter M, Hamadani JD, Nermell B, Huda SN, Yunus M, Rahman M, Grantham-McGregor SM. Effect of arsenic exposure during pregnancy on infant development at 7 months in rural Matlab, Bangladesh. Environ Health Perspect. 2009;117:288–93.

27. Xu L, Yokoyama K, Tian Y, Piao F-Y, Kitamura F, Kida H, Wang P. Decrease in birth weight and gestational age by arsenic among the newborn in Shanghai, China. Nihon Koshu Eisei Zasshi. 2011;58:89–95.

28. Guan H, Piao F, Zhang X, Li X, Li Q, Xu L, Kitamura F, Yokoyama K. Prenatal exposure to arsenic and its effects on fetal development in the general population of Dalian. Biol Trace Elem Res. 2012;149:10–5.

29. Laine JE, Bailey KA, Rubio-Andrade M, Olshan AF, Smeester L, Drobná Z, Herring AH, Stýblo M, García-Vargas GG, Fry RC. Maternal arsenic exposure, arsenic methylation efficiency, and birth outcomes in the Biomarkers of Exposure to ARsenic (BEAR) pregnancy cohort in Mexico. Environ Health Perspect. 2015;123:186–92.

30. Kile ML, Cardenas A, Rodrigues E, Mazumdar M, Dobson C, Golam M, Quamruzzaman Q, Rahman M, Christiani DC. Estimating Effects of Arsenic Exposure During Pregnancy on Perinatal Outcomes in a Bangladeshi Cohort. Epidemiology. 2016;27:173–81.

31. Klemm RDW, Merrill RD, Wu L, Shamim AA, Ali H, Labrique A, Christian P, West KP. Low-birthweight rates higher among Bangladeshi neonates measured during active birth surveillance compared to national survey data. Matern Child Nutr. 2015;11:583–

94.

32. Wilcox AJ, Russell IT. Birthweight and perinatal mortality: II. On weight-specific mortality. Int J Epidemiol. 1983;12:319–25.

33. Hack M, Klein NK, Taylor HG. Long-term developmental outcomes of low birth weight infants. Futur Child. 1995;5:176–96.

34. Lechtig A, Yarbrough C, Delgado H, Habicht JP, Martorell R, Klein RE. Influence of maternal nutrition on birth weight. Am J Clin Nutr. 1975;28:1223–33.

35. Huyck KL, Kile ML, Mahiuddin G, Quamruzzaman Q, Rahman M, Breton C V., Dobson CB, Frelich J, Hoffman E, Yousuf J, et al. Maternal Arsenic Exposure Associated With Low Birth Weight in Bangladesh. J Occup Environ Med. 2007;49:1097–104.

36. Moore VM, Davies MJ. Diet during pregnancy, neonatal outcomes and later health. Reprod Fertil Dev. 2005;17:341–8.

37. Kordas K, Lönnerdal B, Stoltzfus RJ. Interactions between nutrition and environmental exposures: effects on health outcomes in women and children. J Nutr. 2007;137:2794–7.

38. Lowensohn RI, Stadler DD, Naze C. Current Concepts of Maternal Nutrition. Obstet Gynecol Surv. 2016;71:413–26.

39. Budtz-Jørgensen E, Keiding N, Grandjean P, Weihe P. Estimation of health effects of prenatal methylmercury exposure using structural equation models. Environ Health. 2002;1:2.

40. Davidson PW, Strain JJ, Myers GJ, Thurston SW, Bonham MP, Shamlaye CF, Stokes-Riner A, Wallace JMW, Robson PJ, Duffy EM, et al. Neurodevelopmental effects of maternal nutritional status and exposure to methylmercury from eating fish during

pregnancy. Neurotoxicology. NIH Public Access; 2008;29:767–75.

41. Davidson PW, Cory-Slechta DA, Thurston SW, Huang L-S, Shamlaye CF, Gunzler D, Watson G, van Wijngaarden E, Zareba G, Klein JD, et al. Fish consumption and prenatal methylmercury exposure: cognitive and behavioral outcomes in the main cohort at 17 years from the Seychelles child development study. Neurotoxicology. NIH Public Access; 2011;32:711–7.

42. Maiti, Chatterjee. Differential response of cellular antioxidant mechanism of liver and kidney to arsenic exposure and its relation to dietary protein deficiency. Environ Toxicol Pharmacol. 2000;8:227–35.

43. Heck JE, Gamble M V, Chen Y, Graziano JH, Slavkovich V, Parvez F, Baron JA, Howe GR, Ahsan H. Consumption of folate-related nutrients and metabolism of arsenic in Bangladesh. Am J Clin Nutr. 2007;85:1367–74.

44. Rezaul Karim M, Ahmad SA. Nutritional Status among the Children of Age Group 5-14 Years in Selected Arsenic Exposed and Non-Exposed Areas of Bangladesh. J Fam Reprod Heal. 2014;8:161–8.

45. Niedzwiecki MM, Hall MN, Liu X, Slavkovich V, Ilievski V, Levy D, Alam S, Siddique AB, Parvez F, Graziano JH, et al. Interaction of plasma glutathione redox and folate deficiency on arsenic methylation capacity in Bangladeshi adults. Free Radic Biol Med. 2014;73:67–74.

46. Lin P-I, Bromage S, Mostofa M, Allen J, Oken E, Kile M, Christiani D. Associations between Diet and Toenail Arsenic Concentration among Pregnant Women in Bangladesh: A Prospective Study. Nutr 2017, Vol 9, Page 420. Multidisciplinary Digital Publishing Institute; 2017;9:420.

47. Valeri L, VanderWeele TJ. Mediation analysis allowing for exposure–mediator interactions and causal interpretation: Theoretical assumptions and implementation with SAS and SPSS macros. Psychol Methods. 2013;18:137–50.

48. Kile ML, Rodrigues EG, Mazumdar M, Dobson CB, Diao N, Golam M, Quamruzzaman Q, Rahman M, Christiani DC. A prospective cohort study of the association between drinking water arsenic exposure and self-reported maternal health symptoms during pregnancy in Bangladesh. Environ Heal. 2014;13:29.

49. Rodrigues EG, Kile M, Dobson C, Amarasiriwardena C, Quamruzzaman Q, Rahman M, Golam M, Christiani DC. Maternal-infant biomarkers of prenatal exposure to arsenic and manganese. J Expo Sci Environ Epidemiol. 2015;25:639–48.

50. Cubadda F, D'Amato M, Mancini FR, Aureli F, Raggi A, Busani L, Mantovani A. Assessing human exposure to inorganic arsenic in high-arsenic areas of Latium: a biomonitoring study integrated with indicators of dietary intake. Ann Ig. 2015;27:39–51.

51. Karagas MR, Morris JS, Weiss JE, Spate V, Baskett C, Greenberg ER. Toenail samples as an indicator of drinking water arsenic exposure. Cancer Epidemiol Biomarkers Prev. 1996;5:849–52.

52. Chen K-LBL, Amarasiriwardena CJ, Christiani DC. Determination of total arsenic concentrations in nails by inductively coupled plasma mass spectrometry. Biol Trace Elem Res. 1999;67:109–25.

53. Lin P-ID, Bromage S, Mostofa MG, Allen J, Oken E, Kile ML, Christiani DC. Validation of a Dish-Based Semiquantitative Food Questionnaire in Rural Bangladesh. Nutrients. 2017;9:49.

54. Michels KB, Willett WC. Self-administered semiquantitative food frequency

questionnaires: patterns, predictors, and interpretation of omitted items. Epidemiology. NIH Public Access; 2009;20:295–301.

55. Shaheen N, Rahim AT, Banu, Mohiduzzaman Cadi Parvin, Bari L, Tukun B, Mannan M, Bhattacharjee L, Stadlmayr B. Food Composition Table for Bangladesh. Dhaka; 2013.

56. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr. 1997;65:1220S–1228S; discussion 1229S–1231S.

57. Rubin DB, B. D. Estimating causal effects of treatments in randomized and nonrandomized studies. J Educ Psychol. American Psychological Association; 1974;66:688–701.

58. Cottingham KL, Karimi R, Gruber JF, Zens MS, Sayarath V, Folt CL, Punshon T, Morris JS, Karagas MR. Diet and toenail arsenic concentrations in a New Hampshire population with arsenic-containing water. Nutr J. BioMed Central; 2013;12:149.

59. Alam MGM, Snow ET, Tanaka A. Arsenic and heavy metal contamination of vegetables grown in Samta village, Bangladesh. Sci Total Environ. 2003;308:83–96.

60. Das HK, Mitra AK, Sengupta PK, Hossain A, Islam F, Rabbani GH. Arsenic concentrations in rice, vegetables, and fish in Bangladesh: a preliminary study. Environ Int. 2004;30:383–7.

61. Kile ML, Houseman EA, Breton C V, Smith T, Quamruzzaman Q, Rahman M, Mahiuddin G, Christiani DC. Dietary arsenic exposure in bangladesh. Environ Health Perspect. 2007;115:889–93.

62. M. Smith N, Lee R, Heitkemper DT, DeNicola Cafferky K, Haque A, Henderson AK. Inorganic arsenic in cooked rice and vegetables from Bangladeshi households. Sci Total

Environ. 2006;370:294–301.

63. Alava P, Du Laing G, Tack F, De Ryck T, Van De Wiele T. Westernized diets lower arsenic gastrointestinal bioaccessibility but increase microbial arsenic speciation changes in the colon. Chemosphere. 2015;119:757–62.

64. Tseng C-H. A review on environmental factors regulating arsenic methylation in humans. Toxicol Appl Pharmacol. 2009;235:338–50.

65. Steinmaus C, Carrigan K, Kalman D, Atallah R, Yuan Y, Smith AH. Dietary intake and arsenic methylation in a U.S. population. Environ Health Perspect. 2005;113:1153–9.

66. Siega-Riz AM, Adair LS. Biological determinants of pregnancy weight gain in a Filipino population. Am J Clin Nutr. American Society for Nutrition; 1993;57:365–72.

67. Stuebe AM, Oken E, Gillman MW. Associations of diet and physical activity during pregnancy with risk for excessive gestational weight gain. Am J Obstet Gynecol. 2009;201:58.e1-58.e8.

68. Uusitalo U, Arkkola T, Ovaskainen M-L, Kronberg-Kippilä C, Kenward MG, Veijola R, Simell O, Knip M, Virtanen SM. Unhealthy dietary patterns are associated with weight gain during pregnancy among Finnish women. Public Health Nutr. 2009;12:2392.

69. Castro P da S, Castro MBT de, Kac G. [Adherence to dietary recommendations by the Institute of Medicine and the effect on body weight during pregnancy]. Cad Saude Publica. 2013;29:1311–21.

70. Gaillard R, Durmuş B, Hofman A, Mackenbach JP, Steegers EAP, Jaddoe VWV. Risk factors and outcomes of maternal obesity and excessive weight gain during pregnancy. Obesity. 2013;21:1046–55.

71. Changamire FT, Mwiru RS, Msamanga GI, Spiegelman D, Urassa W, Hertzmark E,

Fawzi WW, Peterson KE. Macronutrient and sociodemographic determinants of gestational weight gain among HIV-negative women in Tanzania. Food Nutr Bull. 2014;35:43–50.

72. Tielemans MJ, Garcia AH, Peralta Santos A, Bramer WM, Luksa N, Luvizotto MJ, Moreira E, Topi G, de Jonge EA, Visser TL, et al. Macronutrient composition and gestational weight gain: a systematic review. Am J Clin Nutr. 2016;103:83–99.

73. Bergmann MM, Flagg EW, Miracle-McMahill HL, Boeing H. Energy intake and net weight gain in pregnant women according to body mass index (BMI) status. Int J Obes Relat Metab Disord. 1997;21:1010–7.

74. Ebrahimi F, Shariff ZM, Tabatabaei SZ, Fathollahi MS, Mun CY, Nazari M. Relationship between sociodemographics, dietary intake, and physical activity with gestational weight gain among pregnant women in Rafsanjan City, Iran. J Health Popul Nutr. 2015;33:168–76.

75. Godfrey K, Robinson S, Barker DJ, Osmond C, Cox V. Maternal nutrition in early and late pregnancy in relation to placental and fetal growth. BMJ. BMJ Publishing Group; 1996;312:410–4.

76. Mathews F, Yudkin P, Neil A. Influence of maternal nutrition on outcome of pregnancy: prospective cohort study. BMJ. 1999;319.

77. Andreasyan K, Ponsonby A-L, Dwyer T, Morley R, Riley M, Dear K, Cochrane J. Higher maternal dietary protein intake in late pregnancy is associated with a lower infant ponderal index at birth. Eur J Clin Nutr. 2006;61:498–508.

78. Susser M. Maternal weight gain, infant birth weight, and diet: causal sequences. Am J Clin Nutr. 1991;53:1384–96.

79. Maslova E, Halldorsson TI, Astrup A, Olsen SF. Dietary protein-to-carbohydrate ratio and added sugar as determinants of excessive gestational weight gain: a prospective cohort study. BMJ Open. British Medical Journal Publishing Group; 2015;5:e005839.

80. Kramer MS, Kakuma R. Energy and protein intake in pregnancy. In: Kramer MS, editor. Cochrane Database of Systematic Reviews. Chichester, UK: John Wiley & Sons, Ltd; 2003. p. CD000032.

81. Switkowski KM, Jacques PF, Must A, Kleinman KP, Gillman MW, Oken E. Maternal protein intake during pregnancy and linear growth in the offspring. Am J Clin Nutr. 2016;104:1128–36.

82. Gleason K, Shine JP, Shobnam N, Rokoff LB, Suchanda HS, Ibne Hasan MOS, Mostofa G, Amarasiriwardena C, Quamruzzaman Q, Rahman M, et al. Contaminated turmeric is a potential source of lead exposure for children in rural Bangladesh. J Environ Public Health. 2014;2014:730636.

83. Rees JR, Sturup S, Chen C, Folt C, Karagas MR. Toenail mercury and dietary fish consumption. J Expo Sci Environ Epidemiol. 2007;17:25–30.

84. Islam GMR, Khan FE, Hoque MM, Jolly YN. Consumption of unsafe food in the adjacent area of Hazaribag tannery campus and Buriganga River embankments of Bangladesh: heavy metal contamination. Environ Monit Assess. Springer International Publishing; 2014;186:7233–44.

85. Oku T, Konishi F, Hosoya N. Mechanism of inhibitory effect of unavailable carbohydrate on intestinal calcium absorption. J Nutr. 1982;112:410–5.

86. Hallberg L. Dietary fibre and mineral absorption. Scand J Gastroenterol. Taylor & Francis; 1987;22:66–7.

87. McCallum B, McCallum, Bennett. Relative Asymptotic Bias from Errors of Omission and Measurement. Econometrica. Econometric Society; 1972;40:757–8.

88. Ahmed A. Bangladesh Integrated Household Survey (BIHS) 2011-2012. Washington, D.C.; 2013.

89. Willet W. Food Frequency Methods. Nutritional Epidemiology. 3rd ed. Oxford: Oxford University Press; 2013. p. 71–2.

90. Butt K, Lim K, Bly S, Cargill Y, Davies G, Denis N, Hazlitt G, Morin L, Ouellet A, Salem S. Determination of Gestational Age by Ultrasound. J Obstet Gynaecol Canada. 2014;36:171–81.

91. Rashid DA, Smith LC, Rahman T. Determinants of Dietary Quality: Evidence from Bangladesh. World Dev. 2011;39:2221–31.

92. Misra A, Ray S, Patrikar S. A longitudinal study to determine association of various maternal factors with neonatal birth weight at a tertiary care hospital. Med J Armed Forces India. 2015;71:270–3.

93. Miller TL, Jacobson DL, Somarriba G, Neri D, Kurtz-Vraney J, Graham P, Gillman MW, Landy DC, Siminski S, Butler L, et al. A multicenter study of diet quality on birth weight and gestational age in infants of HIV-infected women. Matern Child Nutr. 2016;

94. Gresham E, Collins CE, Mishra GD, Byles JE, Hure AJ. Diet quality before or during pregnancy and the relationship with pregnancy and birth outcomes: the Australian Longitudinal Study on Women's Health. Public Health Nutr. 2016;19:2975–83.

95. Meinila J, Valkama A, Koivusalo SB, Rönö K, Kautiainen H, Lindström J, Stach-Lempinen B, Eriksson JG, Erkkola M. Association between diet quality measured by the Healthy Food Intake Index and later risk of gestational diabetes—a secondary analysis

of the RADIEL trial. Eur J Clin Nutr. 2017;71:555–7.

96. Potdar RD, Sahariah SA, Gandhi M, Kehoe SH, Brown N, Sane H, Dayama M, Jha S, Lawande A, Coakley PJ, et al. Improving women's diet quality preconceptionally and during gestation: effects on birth weight and prevalence of low birth weight--a randomized controlled efficacy trial in India (Mumbai Maternal Nutrition Project). Am J Clin Nutr. American Society for Nutrition; 2014;100:1257–68.

97. Shapiro ALB, Kaar JL, Crume TL, Starling AP, Siega-Riz AM, Ringham BM, Glueck DH, Norris JM, Barbour LA, Friedman JE, et al. Maternal diet quality in pregnancy and neonatal adiposity: the Healthy Start Study. Int J Obes. 2016;40:1056–62.

98. Shin D, Bianchi L, Chung H, Weatherspoon L, Song WO. Is Gestational Weight Gain Associated with Diet Quality During Pregnancy? Matern Child Health J. 2014;18:1433–43.

99. Opie RS, Neff M, Tierney AC. A behavioural nutrition intervention for obese pregnant women: Effects on diet quality, weight gain and the incidence of gestational diabetes. Aust New Zeal J Obstet Gynaecol. 2016;56:364–73.

100. Guenther PM, Casavale KO, Reedy J, Kirkpatrick SI, Hiza HAB, Kuczynski KJ, Kahle LL, Krebs-Smith SM. Update of the Healthy Eating Index: HEI-2010. J Acad Nutr Diet. NIH Public Access; 2013;113:569–80.

101. Guenther PM, Kirkpatrick SI, Reedy J, Krebs-Smith SM, Buckman DW, Dodd KW, Casavale KO, Carroll RJ. The Healthy Eating Index-2010 is a valid and reliable measure of diet quality according to the 2010 Dietary Guidelines for Americans. J Nutr. 2014;144:399–407.

102. Meinilä J, Valkama A, Koivusalo SB, Stach-Lempinen B, Lindström J, Kautiainen H,

Eriksson JG, Erkkola M. Healthy Food Intake Index (HFII) - Validity and reproducibility in a gestational-diabetes-risk population. BMC Public Health. BioMed Central; 2016;16:680.

103. Fiedler JL, Lividini K. Monitoring population diet quality and nutrition status with household consumption and expenditure surveys: suggestions for a Bangladesh baseline. Food Secur. Springer Netherlands; 2017;9:63–88.

104. Shamim AA, Mashreky SR, Ferdous T, Tegenfeldt K, Roy S, Rahman AKMF, Rashid I, Haque R, Rahman Z, Hossen K, et al. Pregnant Women Diet Quality and Its Sociodemographic Determinants in Southwestern Bangladesh. Food Nutr Bull. 2016;37:14–26.

3.7. Supplementary Materials

Table S3-1. Mediation analysis of the estimated effect[1] (95% CI) of maternal energy and nutrient intake (per s.d change) on GAB (week) through ln-transformed toenail arsenic [ln(μg/g)] stratified by drinking water arsenic level and BMI, with no energy adjustment

	GAB (week)		
Intake level	Natural direct effect (95% CI)	Natural indirect effect (95% CI)	Total effect (95% CI)
All subjects (N=1057)			
Energy	**0.115 (0.007, 0.222)**	-0.001 (-0.012, 0.010)	**0.114 (0.005, 0.222)**
Protein	**-0.664 (-0.786, -0.542)**	-0.013 (-0.041, 0.016)	**-0.676 (-0.795, -0.558)**
Fat	**-0.339 (-0.452, -0.226)**	**-0.022 (-0.042, -0.001)**	**-0.362 (-0.473, -0.250)**
Carbohydrate	**0.471 (0.359, 0.582)**	**0.017 (0.001, 0.034)**	**0.488 (0.377, 0.599)**
Fiber	**-0.216 (-0.331, -0.101)**	**-0.025 (-0.046, -0.005)**	**-0.241 (-0.355, -0.127)**
Drinking water arsenic <50 μg/L (N=833)			
Energy	0.106 (-0.007, 0.218)	0.001 (-0.006, 0.008)	0.107 (-0.006, 0.219)
Protein	**-0.682 (-0.815, -0.550)**	0.015 (-0.004, 0.034)	**-0.667 (-0.798, -0.535)**
Fat	**-0.341 (-0.469, -0.213)**	0.006 (-0.007, 0.020)	**-0.334 (-0.461, 0.020)**
Carbohydrate	**0.420 (0.310, 0.540)**	-0.001 (-0.001, 0.001)	**0.420 (0.300, 0.540)**
Fiber	**-0.193 (-0.320,- 0.065)**	0.005 (-0.009, 0.018)	**-0.187 (-0.314, -0.061)**
Drinking water arsenic ≥50 μg/L (N=224)			
Energy	0.025 (-0.306, 0.356)	0.029 (-0.022, 0.081)	0.054 (-0.277, 0.384)
Protein	**-0.246 (-0.591 -0.098)**	-0.014 (-0.053, 0.025)	**-0.261 (-0.607, -0.085)**
Fat	-0.142 (-0.402, 0.117)	-0.010 (-0.039, 0.020)	-0.153 (-0.413, 0.108)
Carbohydrate	0.367 (-0.095, 0.829)	0.061 (-0.059, 0.183)	0.429 (-0.019, 0.878)
Fiber	-0.057 (-0.353, 0.240)	-0.004 (-0.035, 0.027)	0.061 (-0.359, 0.237)
Normal BMI (18.5≤BMI<30.0) (n=751)			
Energy	0.126 (-0.001, 0.253)	-0.001 (-0.012, 0.009)	0.125 (-0.002, 0.252)
Protein	**-0.643 (-0.790, -0.500)**	-0.005 (-0.037, 0.029)	**-0.648 (-0.791, -0.510)**
Fat	**-0.332 (-0.466, -0.198)**	-0.015 (-0.038, 0.007)	**-0.347 (-0.480, -0.215)**
Carbohydrate	**0.467 (0.337, 0.598)**	0.011 (-0.006, 0.028)	**0.479 (0.350, 0.608)**
Fiber	**-0.226 (-0.359, -0.092)**	-0.017 (-0.038, 0.005)	**-0.243 (-0.375, -0.110)**
Underweight women (BMI<18.5)(n=296)			
Energy	0.015 (-0.199, 0.228)	0.004 (-0.031, 0.039)	0.019 (-0.198, 0.235)
Protein	**-0.719 (-0.947, -0.491)**	-0.037 (-0.096, 0.021)	**-0.757 (-0.978, -0.535)**
Fat	**-0.376 (-0.595, -0.156)**	-0.040 (-0.086, 0.006)	**-0.415 (-0.634, -0.197)**
Carbohydrate	**0.410 (0.180, 0.641)**	0.043 (-0.001, 0.093)	**0.455 (0.226, 0.684)**
Fiber	-0.217 (-0.453, 0.020)	-0.051 (-0.106, 0.003)	**-0.268 (-0.503, -0.033)**

Abbreviations: CI, confidence interval. Bold letter indicates $p<0.01$

[1] The natural direct effect, natural indirect effect, and total effects reflect the change in gestational age at birth, GAB (week) gestational weight gain, GWG (lb/week), or birth weight, BW (gram) per s.d increase in intake and are measured based on intake change from mean minus 1 s.d to mean. Model was adjusted for BMI at the time of enrollment, exposure to environmental tobacco smoke, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, and hour spend cooking over open fire every day.

Table S3-2. Mediation analysis of the estimated effect[1] (95% CI) of maternal energy and nutrient intake (per s.d change) on GWG (lb/week) through ln-transformed toenail arsenic [ln(μg/g)] stratified by drinking water arsenic level and BMI, with no energy adjustment

	GWG (lb/week)		
Intake level	Natural direct effect (95% CI)	Natural indirect effect (95% CI)	Total effect (95% CI)
All subjects (N=1057)			
Energy	0.003 (-0.014, 0.019)	0.001 (-0.001, 0.001)	0.002 (-0.014, 0.019)
Protein	**-0.047 (-0.066, -0.028)**	-0.003 (-0.008, 0.001)	**-0.050 (-0.069, -0.031)**
Fat	**-0.021 (-0.044, -0.040)**	-0.003 (-0.007, 0.000)	**-0.025 (-0.042, -0.008)**
Carbohydrate	**0.026 (0.009, 0.043)**	0.003 (-0.001, 0.005)	**0.028 (0.011, 0.045)**
Fiber	**-0.026 (-0.043, -0.009)**	-0.003 (-0.006, 0.000)	**-0.029 (-0.046, -0.012)**
Drinking water arsenic <50 μg/L *(N=833)*			
Energy	0.001 (-0.017, 0.018)	-0.000 (-0.002, 0.001)	0.001 (-0.018, 0.018)
Protein	**-0.046 (-0.068, -0.024)**	-0.001 (-0.003, 0.002)	**-0.047 (-0.069, -0.025)**
Fat	-0.019 (-0.040, 0.001)	-0.001(-0.003, 0.001)	-0.020 (-0.041, 0.003)
Carbohydrate	**0.020 (0.001, 0.039)**	0.001 (-0.001, 0.001)	**0.020 (0.001, 0.039)**
Fiber	**-0.030 (-0.050, -0.001)**	-0.001 (-0.003, 0.001)	**-0.031 (-0.051, -0.010)**
Drinking water arsenic ≥50 μg/L *(N=224)*			
Energy	-0.016 (-0.057, 0.026)	0.001 (-0.005, 0.005)	-0.016 (-0.057, 0.025)
Protein	-0.019 (-0.063, 0.024)	0.001 (-0.002, 0.003)	-0.020 (-0.062, 0.024)
Fat	-0.022 (-0.055, 0.010)	0.002 (-0.001, 0.002)	-0.022 (-0.055, 0.010)
Carbohydrate	-0.002 (-0.060, 0.056)	0.001 (-0.016, 0.013)	-0.004 (-0.060, 0.053)
Fiber	-0.010 (-0.047, 0.027)	0.001 (-0.001, 0.001)	-0.001 (-0.050, 0.027)
Normal BMI (18.5≤BMI<30.0) (n=751)			
Energy	0.003 (-0.016, 0.021)	-0.001 (-0.002, 0.001)	0.002 (-0.016, 0.021)
Protein	**-0.040 (-0.062, -0.017)**	-0.003 (-0.009, 0.002)	**-0.043 (-0.065, -0.021)**
Fat	-0.012 (-0.032, 0.008)	-0.003 (-0.007, 0.001)	-0.015 (-0.035, 0.005)
Carbohydrate	0.020 (-0.001, 0.040)	0.002 (-0.001, 0.005)	**0.022 (0.002, 0.042)**
Fiber	**-0.020 (-0.040, -0.001)**	-0.003 (-0.005, 0.001)	**-0.023 (-0.042,-0.003)**
Underweight women (BMI<18.5) (n=296)			
Energy	-0.003 (-0.037, 0.030)	0.001 (-0.003, 0.001)	0.003 (-0.037, 0.031)
Protein	**-0.058 (-0.097, -0.021)**	-0.005 (-0.014, 0.005)	**-0.063 (-0.100, -0.027)**
Fat	**-0.044 (-0.079, -0.008)**	-0.004 (-0.010, 0.002)	**-0.050 (-0.082, -0.013)**
Carbohydrate	0.033 (-0.004, 0.070)	0.005 (-0.002, 0.012)	**0.038 (0.001, 0.074)**
Fiber	**-0.048 (-0.085, -0.010)**	-0.005 (-0.012, 0.003)	**-0.053 (-0.089, -0.016)**

Abbreviations: CI, confidence interval. Bold letter indicates p<0.01

[1] The natural direct effect, natural indirect effect, and total effects reflect the change in gestational age at birth, GAB (week) gestational weight gain, GWG (lb/week), or birth weight, BW (gram) per s.d increase in intake and are measured based on intake change from mean minus 1 s.d to mean. Model was adjusted for BMI at the time of enrollment, exposure to environmental tobacco smoke, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, and daily hours spent cooking over an open fireevery day.

Table S3-3. Mediation analysis of the estimated effect[1] (95% CI) of maternal energy and nutrient intake (per s.d change) on BW (gram) through ln-transformed toenail arsenic [ln(μg/g)] stratified by drinking water arsenic level and BMI, with no energy adjustment

	BW (gram)		
Intake level	Natural direct effect (95% CI)	Natural indirect effect (95% CI)	Total effect (95% CI)
All subjects (N=1057)			
Energy	14.16 (-9.06, 37.36)	0.035 (-0.357, 0.426)	14.19 (-9.05, 37.40)
Protein	-22.82 (-50.39, 4.75)	2.90 (-3.59, 9.39)	-19.92 (-46.74, 6.90)
Fat	-0.14 (-24.87, 24.59)	1.06 (-3.06, 5.13)	0.92 (-23.48, 25.32)
Carbohydrate	23.89 (-0.786, 48.57)	-1.33 (-4.76, 2.10)	22.56 (-1.89, 47.01)
Fiber	15.44 (-9.53, 40.41)	0.64 (-3.34, 4.63)	16.08 (-8.56, 40.73)
Drinking water arsenic <50 μg/L (N=833)			
Energy	7.07 (-16.39, 30.52)	0.63 (-0.91, 2.18)	7.70 (-15.73, 31.13)
Protein	**-36.56 (-65.70, -7.43)**	2.47 (-1.54, 6.48)	**-34.09 (-36.1, -5.18)**
Fat	-11.39 (-38.37, 15.59)	1.48 (-1.48, 4.43)	-9.91 (-36.76, 16.94)
Carbohydrate	21.38 (-3.34, 46.10)	-0.09 (-0.96, 0.77)	21.28 (-3.45, 46.01)
Fiber	4.40 (-22.17, 30.98)	1.29 (-1.59, 4.20)	5.70 (-20.75, 32.14)
Drinking water arsenic ≥50 μg/L (N=224)			
Energy	48.57 (-33.98, 131.11)	-0.65 (-10.47, 9.17)	47.92 (-34.05, 129.88)
Protein	55.18 (-31.17, 141.54)	-0.20 (-5.24, 4.83)	54.98 (-31.22, 141.20)
Fat	51.59 (-13.10, 116.29)	-0.17 (-3.72, 3.39)	51.43 (-13.17, 116.03)
Carbohydrate	5.80 (-110.55, 112.15)	-0.28 (-29.62, 29.10)	5.52 (-107.07, 118.11)
Fiber	**75.53 (2.02, 149.05)**	-0.28 (-1.35, 1.30)	**75.5 (2.00, 149.00)**
Normal BMI (18.5≤BMI<30.0) (n=751)			
Energy	21.44 (-5.67, 48.56)	0.14 (-0.72, 1.00)	21.58 (-5.54, 48.71)
Protein	-6.86 (-39.79, 26.01)	3.31 (-4.20, 10.82)	-3.55 (-35.64, 28.55)
Fat	13.45 (-15.68, 42.59)	1.56 (-3.25, 6.37)	15.01 (-13.74, 43.77)
Carbohydrate	23.88 (-4.85, 52.62)	-1.79 (-5.46, 1.88)	22.10 (-6.44, 50.63)
Fiber	25.90 (-2.83, 54.62)	1.15 (-3.16, 5.47)	27.06 (-1.36, 55.47)
Underweight women (BMI<18.5) (n=296)			
Energy	-14.56 (-61.04, 31.91)	0.19 (-1.63, 2.02)	-14.36 (-60.87, 32.14)
Protein	**-64.60 (-117.13, -12.08)**	0.001 (-12.82, 12.82)	**-64.61 (-115.54, -13.67)**
Fat	-36.94 (-85.47, 11.59)	-1.42 (-9.34, 6.51)	-38.56 (-86.26, 9.54)
Carbohydrate	14.92 (-36.43, 66.26)	2.25 (-6.86, 11.37)	17.17 (-33.41, 67.75)
Fiber	-30.78 (-82.53, 20.96)	-1.82 (-11.23, 7.59)	-32.60 (-83.5, 18.31)

Abbreviations: CI, confidence interval. Bold letter indicates p<0.01

[1] The natural direct effect, natural indirect effect, and total effects reflect the change in gestational age at birth, GAB (week) gestational weight gain, GWG (lb/week), or birth weight, BW (gram) per s.d increase in intake and are measured based on intake change from mean minus 1 s.d to mean. Model was adjusted for BMI at the time of enrollment, exposure to environmental tobacco smoke, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, and daily hours spent cooking over an open fire every day.

Table S3-4. Mediation analysis of the estimated effect[1] (95% CI) of maternal energy and nutrient intake (per s.d change) on gestational age at birth, GAB (week) through ln-transformed toenail arsenic [ln(μg/g)] stratified by drinking water arsenic level and BMI, with energy adjustment[2]

	GAB (week)		
Intake level	Natural direct effect (95% CI)	Natural indirect effect (95% CI)	Total effect (95% CI)
All subjects (N=1057)			
Protein	**-0.873 (-0.996, -0.750)**	0.003 (-0.030, 0.035)	**-0.870 (-0.989, -0.751)**
Fat	**-0.558 (-0.673, -0.443)**	-0.017 (-0.041, 0.007)	**-0.575 (-0.687, -0.462)**
Carbohydrate	**0.787 (0.665, 0.908)**	0.002 (-0.030, 0.034)	**0.789 (0.671, 0.906)**
Fiber	**-0.465 (-0.586, -0.645)**	-0.022 (-0.049, 0.005)	**-0.488 (-0.605, -0.370)**
Drinking water arsenic <50 μg/L *(N=833)*			
Protein	**-0.892 (-1.026, -0.759)**	0.016 (-0.003, 0.034)	**-0.877 (-1.009, -0.744)**
Fat	**-0.555 (-0.683, -0.427)**	0.006 (-0.006, 0.018)	**-0.549 (-0.677, -0.421)**
Carbohydrate	**0.794 (0.660, 0.927)**	-0.013 (-0.030, 0.004)	**0.780 (0.648, 0.913)**
Fiber	**-0.449 (-0.584, -0.314)**	0.006 (-0.007, 0.018)	**-0.443 (-0.578, -0.309)**
Drinking water arsenic ≥50 μg/L *(N=224)*			
Protein	-0.411 (-0.823, 0.002)	0.034 (-0.099, 0.031)	**-0.445 (-0.855, -0.034)**
Fat	-0.265 (-0.572, 0.041)	-0.029 (-0.082, 0.025)	-0.294 (-0.598, 0.102)
Carbohydrate	0.386 (-0.001, 0.771)	0.037 (-0.034, 0.108)	**0.423 (0.041, 0.804)**
Fiber	-0.116 (-0.443, 0.211)	-0.030 (-0.083, 0.023)	-0.146 (-0.472, 0.180)
Normal BMI (18.5≤BMI<30.0) (n=751)			
Protein	**-0.857 (-1.005, -0.709)**	-0.010 (-0.027, 0.047)	**-0.847 (-0.990, -0.704)**
Fat	**-0.553 (-0.688, -0.418)**	-0.009 (-0.036, 0.018)	**-0.562 (-0.695, -0.430)**
Carbohydrate	**0.778 (0.634, 0.922)**	-0.007 (-0.043, 0.029)	**0.771 (0.632, 0.910)**
Fiber	**-0.478 (-0.615, -0.341)**	-0.012 (-0.039, 0.015)	**-0.489 (-0.624, -0.355)**
Underweight women (BMI<18.5)(n=296)			
Protein	**-0.929 (-1.168, -0.691)**	-0.002 (-0.093, 0.046)	**-0.953 (-1.181, -0.724)**
Fat	**-0.554 (-0.786, -0.321)**	-0.045 (-0.102, 0.012)	**-0.599 (-0.824, -0.371)**
Carbohydrate	**0.810 (0.567, 1.053)**	0.032 (-0.039, 0.104)	**0.842 (0.610, 1.075)**
Fiber	**-0.398 (-0.665, -0.131)**	-0.070 (-0.151, 0.011)	**-0.468 (-0.725, -0.211)**

Abbreviations: CI, confidence interval. Bold letter indicates p<0.01

[1] The natural direct effect, natural indirect effect, and total effects reflect the change in gestational age at birth, GAB (week) gestational weight gain, GWG (lb/week), or birth weight, BW (gram) per s.d increase in intake and are measured based on intake change from mean minus 1 s.d to mean. Model was adjusted for BMI at the time of enrollment, exposure to environmental tobacco smoke, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, and daily hours spent cooking over an open fire every day.

[2] Intake level and toenail arsenic level adjusted for energy using the residual method (except for energy)

Table S3-5. Mediation analysis of the estimated effect[1] (95% CI) of maternal energy and nutrient intake (per s.d. change) on gestational weight gain, GWG (lb/week) through ln-transformed toenail arsenic [ln(μg/g)] stratified by drinking water arsenic level and BMI, with energy adjustment[2]

	GWG (lb/week)		
Intake level	Natural direct effect (95% CI)	Natural indirect effect (95% CI)	Total effect (95% CI)
All subjects (N=1057)			
Protein	**-0.058 (-0.078, -0.038)**	-0.003 (-0.008, 0.003)	**-0.061 (-0.080, -0.041)**
Fat	**-0.031 (-0.049, -0.013)**	-0.003 (-0.007, 0.001)	**-0.035 (-0.052, -0.017)**
Carbohydrate	**0.050 (0.031, 0.069)**	0.003 (-0.002, 0.008)	**0.053 (0.034, 0.071)**
Fiber	**-0.042 (-0.061, -0.024)**	-0.003 (-0.007, 0.001)	**-0.046 (-0.064, -0.028)**
Drinking water arsenic <50 μg/L (N=833)			
Protein	**-0.056 (-0.079, -0.033)**	-0.001 (-0.003, 0.002)	**-0.056 (-0.079, -0.033)**
Fat	**-0.026 (-0.047, -0.005)**	-0.001 (-0.003, 0.001)	**-0.027 (-0.048, -0.006)**
Carbohydrate	**0.047 (0.025, 0.070)**	0.001 (-0.002, 0.003)	**0.048 (0.025, 0.070)**
Fiber	**-0.048 (-0.070, -0.026)**	-0.001 (-0.003, 0.001)	**-0.049 (-0.070, -0.027)**
Drinking water arsenic ≥50 μg/L (N=224)			
Protein	-0.020 (-0.072, 0.032)	0.001 (-0.006, 0.008)	-0.019 (-0.071, 0.032)
Fat	-0.029 (-0.067, 0.009)	0.001 (-0.005, 0.007)	-0.028 (-0.066, 0.010)
Carbohydrate	0.026 (-0.022, 0.074)	-0.002 (-0.010, 0.007)	0.024 (-0.024, 0.072)
Fiber	-0.002 (-0.044, 0.038)	-0.001 (-0.005, 0.006)	-0.002 (-0.043, 0.038)
Normal BMI (18.5≤BMI<30.0) (n=751)			
Protein	**-0.049 (-0.073, -0.026)**	-0.003 (-0.009, 0.009)	**-0.052 (-0.075, -0.029)**
Fat	**-0.018 (-0.039, -0.003)**	-0.004 (-0.008, 0.001)	**-0.022 (-0.042, -0.001)**
Carbohydrate	**0.038 (0.015, 0.061)**	0.003 (-0.002, 0.009)	**0.042 (0.019, 0.064)**
Fiber	**-0.033 (-0.054, -0.012)**	-0.003 (-0.007, 0.001)	**-0.036 (-0.056, -0.015)**
Underweight women (BMI<18.5) (n=296)			
Protein	**-0.072 (-0.113, -0.032)**	-0.004 (-0.016, 0.008)	**-0.077 (-0.116, -0.038)**
Fat	**-0.060 (-0.097, -0.022)**	-0.005 (-0.013, 0.004)	**-0.064 (-0.101, -0.027)**
Carbohydrate	**0.074 (0.034, 0.115)**	0.004 (-0.008, 0.016)	**0.078 (0.040, 0.117)**
Fiber	**-0.078 (-0.120, -0.036)**	-0.004 (-0.016, 0.008)	**-0.082 (-0.123, -0.042)**

Abbreviations: CI, confidence interval. Bold letter indicates p<0.01

[1] The natural direct effect, natural indirect effect, and total effects reflect the change in gestational age at birth, GAB (week) gestational weight gain, GWG (lb/week), or birth weight, BW (gram) per s.d increase in intake and are measured based on intake change from mean minus 1 s.d to mean. Model was adjusted for BMI at the time of enrollment, exposure to environmental tobacco smoke, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, and daily hours spent cooking over an open fire every day.

[2] Intake level and toenail arsenic level adjusted for energy using the residual method (except for energy)

Table S3-6. Mediation analysis of the estimated effect[1] (95% CI) of maternal energy and nutrient intake (per s.d. change) on birth weight, BW (gram) through ln-transformed toenail arsenic [ln(μg/g)] stratified by drinking water arsenic level and BMI, with energy adjustment[2]

	GW (gram)		
Intake level	Natural direct effect (95% CI)	Natural indirect effect (95% CI)	Total effect (95% CI)
All subjects (N=1057)			
Protein	**-36.74 (-65.5, -7.95)**	4.40 (-3.23, 12.02)	**-32.34 (-60.14, -4.54)**
Fat	-12.94 (-38.70, 12.81)	1.85 (-3.43, 7.13)	-11.09 (-36.31, 14.12)
Carbohydrate	23.59 (-4.46, 51.54)	-3.40 (-10.75, 3.95)	20.19 (-6.91, 47.28)
Fiber	6.48 (-20.10, 33.06)	1.14 (-4.69, 6.96)	7.61 (-18.33, 33.55)
Drinking water arsenic <50 μg/L (N=833)			
Protein	**-48.83 (-79.17, 18.49)**	2.39 (-1.43, 6.21)	**-46.44 (-76.60, 16.28)**
Fat	-22.21 (-49.98, 5.55)	1.26 (-1.26, 3.79)	-20.95 (-48.64, 6.74)
Carbohydrate	**36.65 (6.80, 66.49)**	-2.09 (-5.69, 1.50)	**34.56 (4.87, 64.24)**
Fiber	-2.83 (-31.63, 25.96	1.23 (-1.46, 3.92)	-1.60 (-30.30, 27.10)
Drinking water arsenic ≥50 μg/L (N=224)			
Protein	54.63 (-49.34, 158.60)	-1.12 (-15.00, 12.75)	53.51 (-49.55, 156.56)
Fat	58.72 (-18.16, 135.59)	-1.49 (-13.26, 10.28)	57.22 (-18.77, 133.21)
Carbohydrate	-83.25 (-180.01, 13.51)	2.45 (-13.64, 18.54)	-80.81 (-176.26, 14.65)
Fiber	79.86 (-1.36, 161.08)	-1.48 (-12.17, 9.22)	78.38 (-2.16, 158.92)
Normal BMI (18.5≤BMI<30.0) (n=751)			
Protein	-22.87 (-57.22, 11.48)	4.73 (-3.95, 13.42)	-18.13 (-51.42, 15.15)
Fat	-1.61 (-31.78, 28.56)	2.37 (-3.63, 8.37)	0.76 (-28.83, 30.36)
Carbohydrate	9.87 (-23.22, 42.95)	-3.77 (-12.07, 4.52)	6.09 (-25.97, 38.16)
Fiber	13.99 (-16.23, 44.22)	1.77 (-4.17, 7.71)	15.76 (-13.89, 45.41)
Underweight women (BMI<18.5) (n=296)			
Protein	**-73.14 (-129.74, -16.55)**	1.36 (15.04, 17.77)	**-71.78 (-125.95, -17.61)**
Fat	-39.43 (-91.80, 12.94)	-1.48 (-13.16, 10.20)	-40.91 (-91.97, 10.14)
Carbohydrate	**59.70 (3.25, 116.16)**	-0.28 (-6.69, 16.12)	**59.43 (5.41, 113.44)**
Fiber	-31.97 (-90.83, 26.89)	-2.26 (-8.97, 14.43)	-34.22 (-90.69, 22.23)

Abbreviations: CI, confidence interval. Bold letter indicates p<0.01

[1] The natural direct effect, natural indirect effect, and total effects reflect the change in gestational age at birth, GAB (week) gestational weight gain, GWG (lb/week), or birth weight, BW (gram) per s.d increase in intake and are measured based on intake change from mean minus 1 s.d to mean. Model was adjusted for BMI at the time of enrollment, exposure to environmental tobacco smoke, age, education level, household income level, newborn sex, birth delivery location, birth delivery type, physical activity level during pregnancy, and daily hours spent cooking over an open fire every day.

[2] Intake level and toenail arsenic level adjusted for energy using the residual method (except for energy)

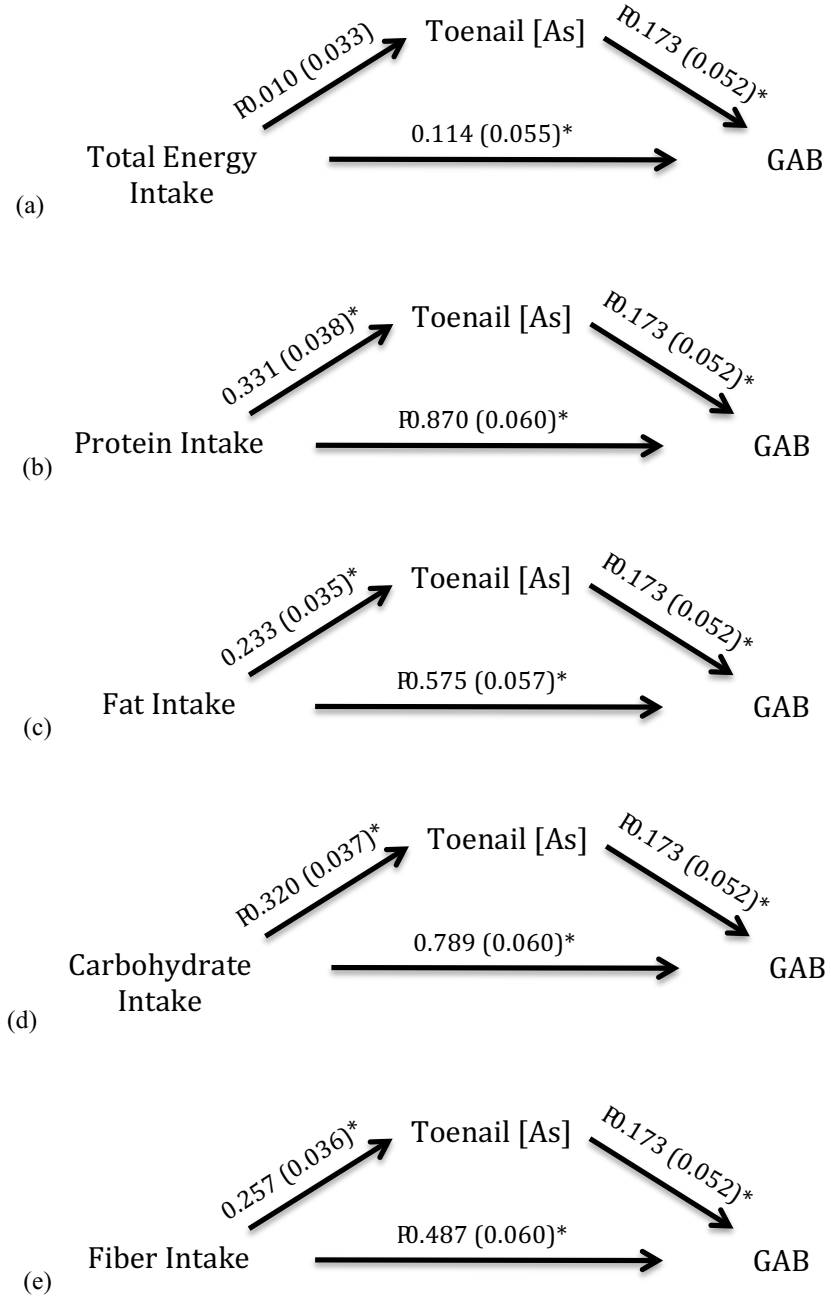

Figure S3-1. Partial regression coefficients on the associations between maternal diet, arsenic exposure and GAB, controlling for potential confounders with energy adjustment. *p<0.05

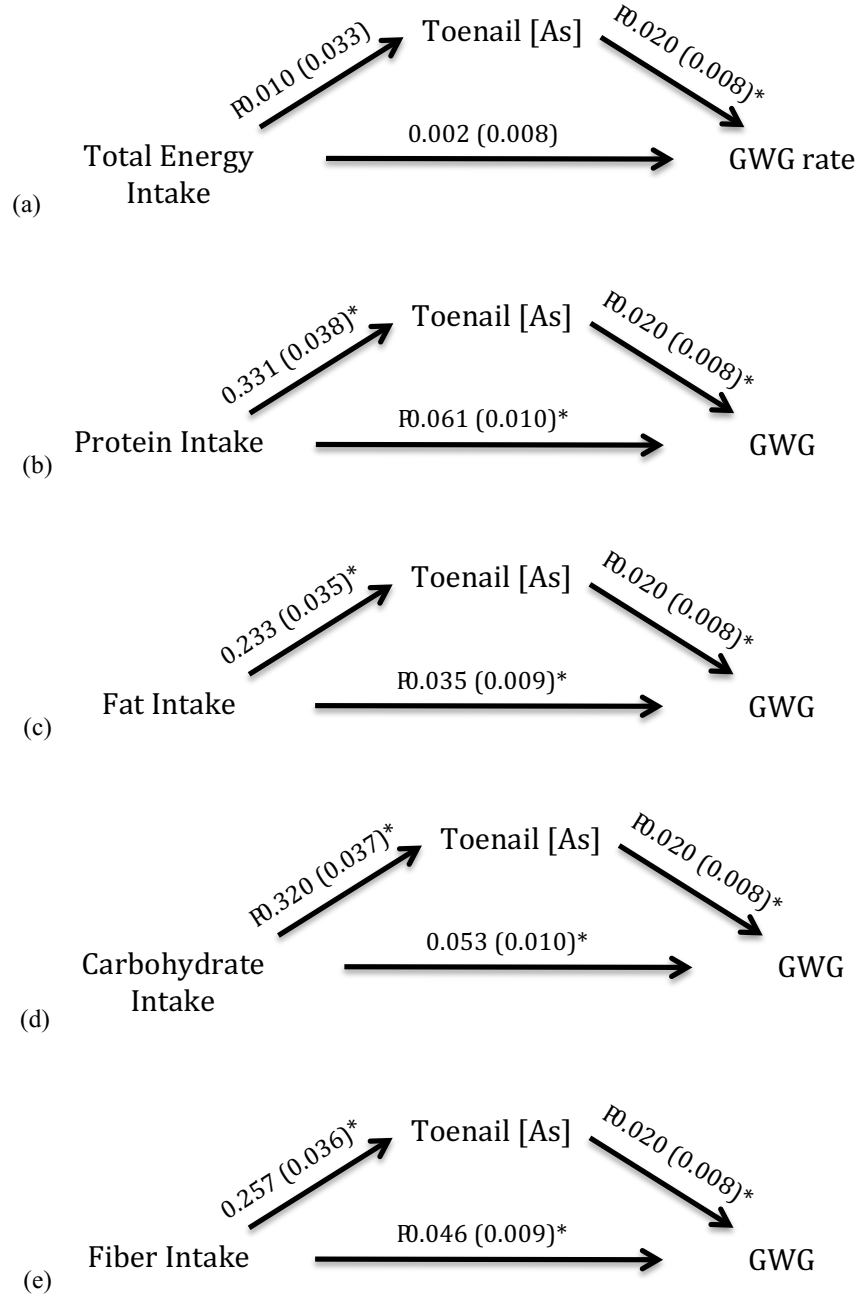

Figure S3-2. Partial regression coefficients on the associations between maternal diet, arsenic exposure and GWG, controlling for potential confounders with energy-adjustment. *p<0.05

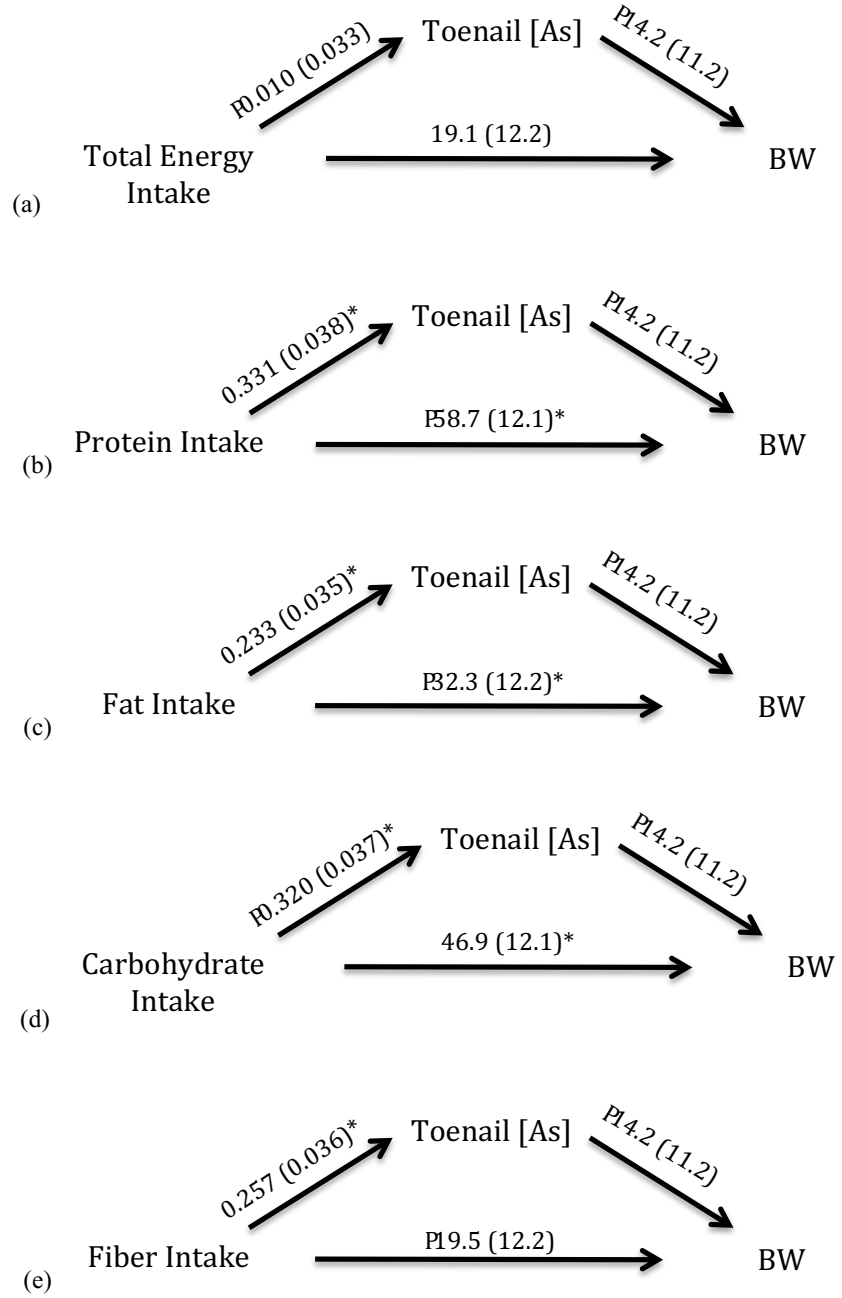

Figure S3-3. Partial regression coefficients on the associations between maternal diet, arsenic exposure and BW, controlling for potential confounders with energy-adjustment. *p<0.05

Summary and Conclusion

The dissertation provided careful assessment of the validity of the dietary instrument developed to assess the habitual diet in Bangladesh and analyzed and converted data on food intakes into nutrient intakes for a large birth cohort in Bangladesh. The dissertation also conducted comprehensive investigation of the effect of diet and nutrition intake on arsenic exposure and birth outcomes among pregnant women in Bangladesh.

In Chapter 1, the validity of the 42-item dish-based semi-quantitative FFQ was assessed by comparing with two 3-day food diaries using Pearson's and Spearman's correlation, paired *t*-test, percent difference, cross-classification, weighted Kappa, and Bland–Altman analysis. Results showed that this dish-based FFQ provided adequate validity to assess and rank long-term dietary intake in rural Bangladesh for most food groups and nutrients, and should be useful for studying dietary-disease relationships.

Chapter 2 evaluated the relationship between long-term dietary habits, as captured by the dish-based FFQ, and total arsenic concentration in toenail clippings among pregnant women in the Bangladesh. Toenail As was not significantly associated with consumption of plain rice as hypothesized. However, toenail As was positively associated with consumption of several vegetable, fish and meat items and was negatively associated with consumption of fried bread, fruits, and milk based food items. The results generated several interesting future research directions, including the change of arsenic metabolism during pregnancy at different level of arsenic exposure, and the interaction between dietary composition and arsenic absorption.

Chapter 3 explored the relationship between diet, arsenic exposure and birth outcomes, including birth weight, gestational age at birth, and gestational weight gain, using a causal

mediation analysis. Although there was not significant mediation by arsenic in the association between maternal diet and birth weight in this study population, higher toenail arsenic associated with higher absolute fat and fiber intake significantly mediated the reduction of gestational age, while lower toenail arsenic associated with higher absolute carbohydrate intake significantly mediate the increase of gestational age. The study supported the hypothesis that absolute intake of arsenic mediates the effect of maternal diet on gestational age.

This dissertation not only provided information on the characteristics of maternal diets among pregnant women in Bangladesh, but also evaluated the association between diet and arsenic exposure and the mediating effect of chronic arsenic exposure on the association between maternal diet and birth outcomes. While the intakes of certain food dishes, including those in the meat, fish and vegetable categories, were associated with higher toenail arsenic levels; intakes of fruits, milk-based dishes, and desserts were associated with lower toenail arsenic level. Future research on the effect of dietary composition and arsenic bioavailability in the body would be interesting. Result of the dissertation showed that in terms of arsenic accumulation in the toenail, the total quantity of arsenic was more important than arsenic in proportion to total energy intake and body size. In terms of the mediating effects contributed by arsenic from diet on birth outcomes, similar result was also noted where the absolute amount of arsenic played a more important role in affecting birth outcome rather than the percent of arsenic in proportion to total energy intake and body size. The study also suggested that individual variation in arsenic methylation capacity may affect the arsenic accumulation and arsenic toxicity, and future research on the association between diet and arsenic methylation capacity will be interesting and can help fill the gap in the understanding of the interaction between arsenic and nutrition.